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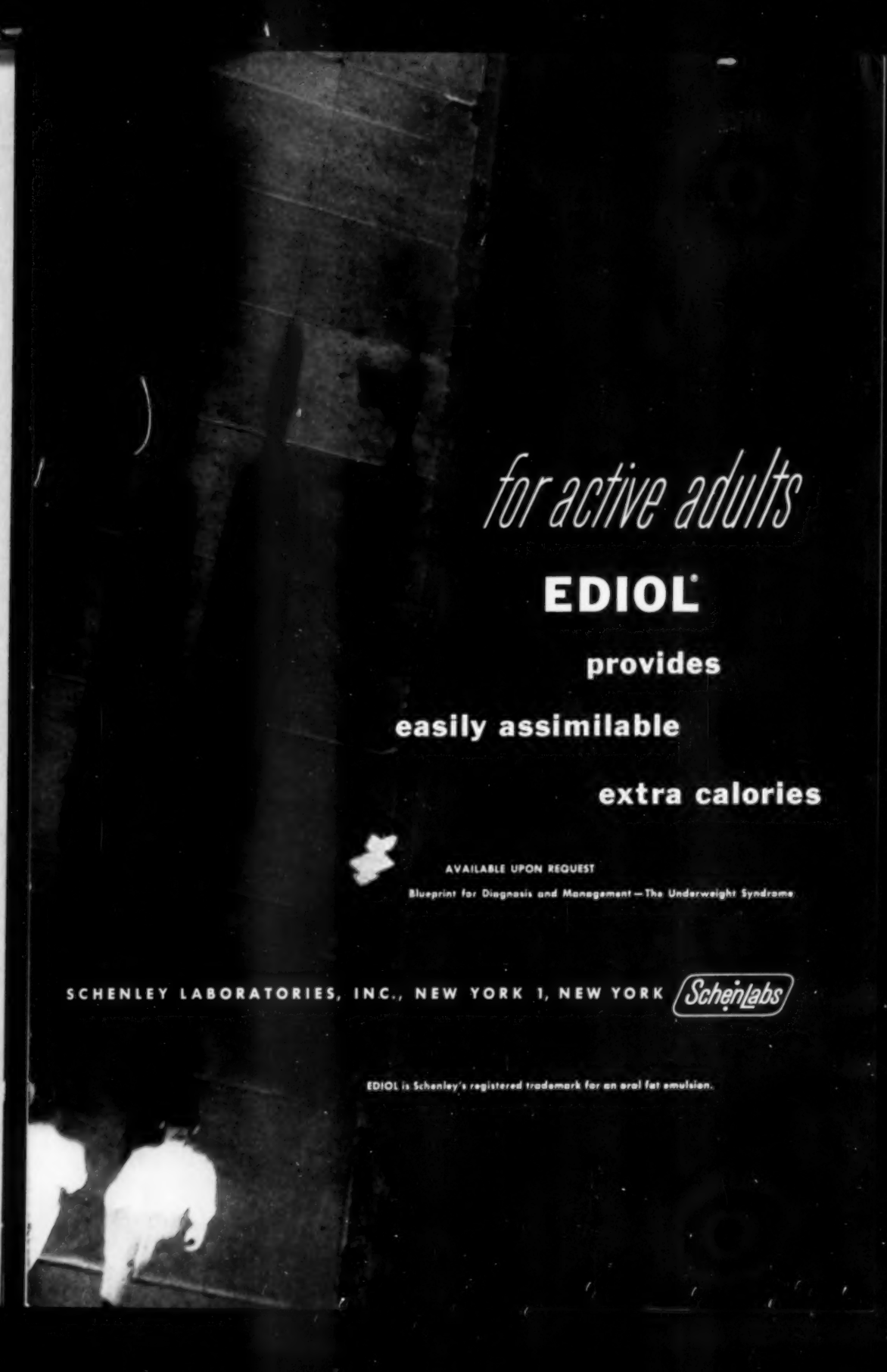
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Evaluation by blood analyses shows that protein needs of the tuberculous patient increase markedly with advancing extent and duration of the disease.[1,2]

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This low serum albumin concentration has been interpreted to reflect "a low protein intake as well as a retarded or failing support with protein released from protein reserves."[2] Unless the low level of serum albumin is corrected by enhanced protein nutrition, it "eventually means a low hemoglobin level and a low total red cell count with cells that have a normal color index."[2]

To satisfy the increased protein needs, 120 grams of protein or more should be supplied in the daily diet.[2] Meat and other high protein foods of animal origin, appetizingly served, assist in getting patients with depressed appetite to eat the amount of food needed to derive the required protein.

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2. Getz, H. R.: Problems in Feeding the Tuberculous Patient, J. Am. Dietet. A. *30*:17 (Jan.) 1954.
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4. Seibert, F. B.; Seibert, M. V.; Atno, A. J., and Campbell, H. W.: Variation in Protein and Polysaccharide Content of Sera in Chronic Diseases, Tuberculosis, Sarcoidosis, and Carcinoma, J. Clin. Invest. *26*:90 (Jan.) 1947.

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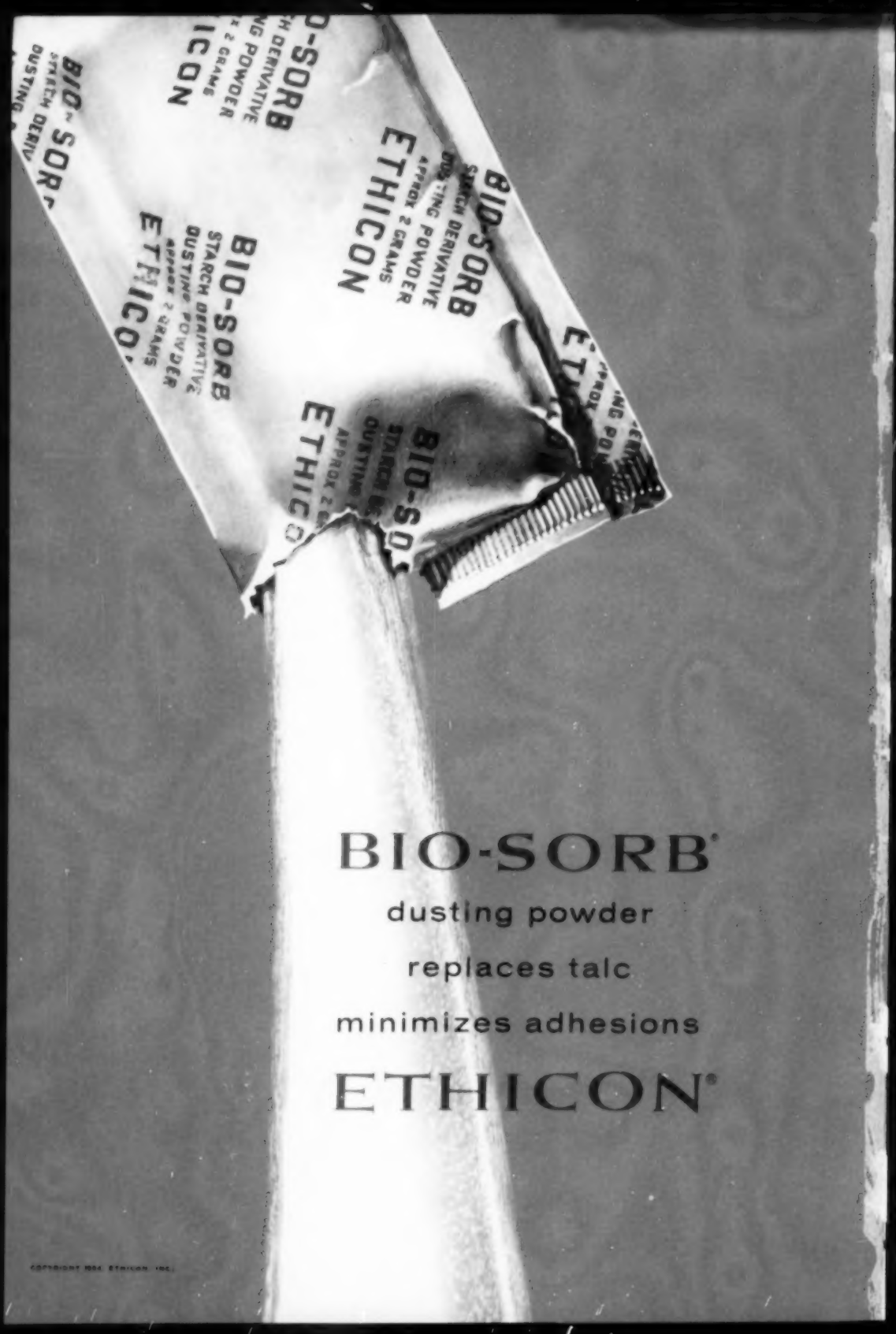
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THE LATE EMERGENCE OF M. TUBERCULOSIS IN LIQUID CULTURES OF PULMONARY LESIONS RESECTED FROM HUMANS[1,2]

GLADYS L. HOBBY, OSCAR AUERBACH, TULITA F. LENERT, MAURICE J. SMALL, AND JOHN V. COMER

(Received for publication April 8, 1954)

INTRODUCTION

A number of years have elapsed since the early studies which established many of the technical methods used today in the field of microbiology. Throughout this period, the microbiologist has been plagued, and often intrigued, by his frequent inability to cultivate in artificial media microorganisms which have become adapted to life within the host. The problem posed by D'Esopo and his associates in 1951 (2), therefore, although of recognized clinical importance, offers a special challenge and stimulus to those interested in the metabolic activities of microorganisms.

In a unique study conducted during the past five years, D'Esopo and his associates (2, 3) demonstrated that tubercle bacilli present in "closed" necrotic lesions after long-term chemotherapy in most instances cannot be cultivated *in vitro* by standard microbiologic techniques. This observation was confirmed by others (4), who likewise used conventional cultural methods, and by Steenken and Wolinsky (5), who utilized more varied and specialized procedures for the purpose. The conclusion was reached that these organisms "behave as if they are dead" (6).

The question has been raised, however, whether or not the techniques used were adequate to prove conclusively the nonviability of microorganisms (6). In recent years, Dubos, Hirsch, and their associates (7–20), Canetti (21), and others (22) have presented evidence to suggest that certain physicochemical factors within necrotic tuberculous lesions may be such as to suppress the metabolic processes of residual bacilli lying therein. Low oxygen tension exists in

[1] From the Research Division of Chas. Pfizer & Co., Inc., Brooklyn, New York; and the Veterans Administration Hospital, East Orange, New Jersey.

[2] Presented in part at the Thirteenth Conference on the Chemotherapy of Tuberculosis, held under the auspices of the Veterans Administration, Army, and Navy, at St. Louis, Missouri, February 8–11, 1954 (1).

the "closed" cavity and at times may render tubercle bacilli noninfectious and nonculturable. Various organic materials, furthermore, accumulate in and around the tuberculous lesion as a result of the anaerobic metabolism of the inflammatory cell and, perhaps, as a result also of the activity of certain lipolytic enzymes released during necrosis. Certain of these substances, e.g., lactic acid and alanine, are capable of enhancing the toxic effects of anaerobiosis upon *Mycobacterium tuberculosis*. In addition, the presence of certain fatty acids in tissue and in necrotic material may suppress the metabolic activities of these microorganisms *in vivo*, while aging of microbial cells undoubtedly occurs in "closed" lesions and is associated with depletion of substances known to be essential for initiation of cellular multiplication *in vitro*. Lastly, four groups of basic compounds possessing antimycobacterial activity must be considered in this regard: (*1*) the basic proteins, protamines, and histones which occur in cell nuclei and are liberated from their union with nucleic acid as a result of necrosis; (*2*) lysozyme, an enzyme which is present in large quantity in leukocytes and which is known to accumulate in areas of inflammation; (*3*) spermine and spermidine, substances which occur in large amount in certain tissues and which, when activated by an enzyme, e.g., spermine oxidase, also present in certain tissues, are capable of inhibiting multiplication of even large inocula of tubercle bacilli; and (*4*) a basic polypeptide isolated from the thymus gland.

There have been frequent descriptions, however, that certain inhibitory effects on cellular metabolism can be completely reversed in favorable circumstances. For example, the *in vitro* inhibitory properties of fatty acids upon tubercle bacilli may be "neutralized" by adsorption onto albumin; the inhibitory effects of lactic acid may be reversed by change of pH and oxygen tension; and the suppressive action of organic acids may be reversed by addition of certain of the keto acids. The universal nature of this phenomenon and the remarkable ability of microorganisms to adapt themselves to adverse environmental conditions are emphasized by the recent observations of Heinmets, Taylor, and Lehman (23). These investigators have demonstrated the presence of viable cells in suspensions of a strain of *Escherichia coli* which have been sterilized by the action of heat, chlorine, Zephiran® chloride, and ethyl alcohol, when such suspensions are incubated with various metabolites of the tricarboxylic acid cycle.

In vivo, the persistence of the spirochete of syphilis, the virus of herpes simplex, or the rickettsia of Brill-Zinsser's disease for long periods of latency within the host, with the ultimate long-delayed development of clinical disease in persons harboring these potentially infectious agents, may be accepted as suggestive evidence that they may revive under appropriate conditions. More concise information in this regard is offered, however, by the observations of Fildes (24). As early as 1927, in a study of the relation of germination of toxin-free tetanus spores to necrosis, he noted that: (*a*) a spore lying free in a necrotic area at once breaks into active vegetation, while another spore lying free in a non-necrotic area remains in a totally passive condition; and (*b*) the oxygen tension may fall to zero in those necrotic areas where germination of spores occurs, while there is a positive tension of oxygen in the tissues in which ger-

mination fails to occur. An analogous situation may well exist for tubercle bacilli which lie within "closed" necrotic lesions, and the development of overt pulmonary tuberculosis in persons whose disease has been arrested for periods of five, ten, or even fifteen years would lend support to this supposition.

Thus, it is within reason to assume that adaptive changes in the enzyme mechanisms within tubercle bacilli may take place *in vivo*, allowing for their survival within the host but preventing their subsequent multiplication *either in vivo or in vitro* until an appropriate reversal mechanism has been imposed. Furthermore, if tubercle bacilli do continue to exist in "closed" caseous lesions in a metabolically suppressed state, changes in the pathologic nature of the lesion (25) and thus in the physicochemical environment of the bacteria conceivably could result in an *in vivo* reversal of those processes responsible for suppression of cellular metabolism. Likewise, alteration in the environment of such microorganisms by removal to an appropriate *in vitro* site might permit them to revive and again multiply.

The present study was undertaken in an attempt to answer the fundamental question: If a tuberculous lesion contains acid-fast bacilli which are visible on microscopy but no organisms can be recovered by conventional cultivation on artificial media or by guinea pig inoculation, is one justified in concluding that the organisms are dead? Microbiologic observations on cultures of resected tuberculous lesions from patients who had had extensive chemotherapy and who had been noninfectious[3] for several months will be reported. Throughout this study, a microbiologic technique designed to neutralize certain of the inhibitory agents present in necrotic tissue and to "dilute out" other potentially toxic components has been used.

MATERIALS AND METHODS

Source of Pulmonary Lesions

A total of 85 necrotic lesions from 40 patients have been cultured to date. These have included filled-in cavities, small necrotic foci which had never liquefied, and open cavities with healing.[4] Due to the long observation period needed for evaluation of results, only 31 lesions from the first 19 patients studied are discussed in the present report.

Eleven of the 19 patients from whom lesions were obtained were "original treatment" cases in which the preoperative course of chemotherapy represented the patients' first exposure to specific antimicrobial therapy. Four of the 19

[3] The term "infectious" is used throughout this report when tubercle bacilli can be demonstrated by microscopy and/or conventional cultural techniques from sputum and/or gastric or tracheal washings of patients. The term "noninfectious," in contrast, is used when, despite repeated attempts, tubercle bacilli can no longer be demonstrated from such sputum and/or gastric or tracheal washings (26).

[4] By open healing is meant the transformation of the cavity wall into hyalinized connective tissue with a complete loss of specific tuberculous elements. The bronchus opening into these cavities is patent, is re-epithelized, and shows a complete loss of specific tuberculous elements.

TABLE 1

SUMMARY OF PREOPERATIVE CLINICAL AND BACTERIOLOGIC DATA ON PATIENTS WITH PULMONARY TUBERCULOSIS

CASE NUMBER	PATIENT	AGE AND SEX	CLINICAL STATUS AT START OF PREOPERATIVE CHEMOTHERAPY	PREOPERATIVE CHEMOTHERAPY		STATUS AT TIME OF SURGERY BY ROENTGENOGRAPHY AND TOMOGRAPHY	PREOPERATIVE DURATION OF NONINFECTIOUSNESS		SURGICAL PROCEDURE
				Regimen	Duration		Microscopy	Culture	
					months		*months*	*months*	
1	J. R.	29M	Moderately advanced, fibrocaseous, with cavitation	Streptomycin (2 × weekly) + PAS daily*	4.5	Marked regression of lesions, LLL; no cavitation, stability for more than 3 months	3	3	Segmental resection, LLL
2	E. H.	47M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily	5.5	Extensive involvement, entire RUL region, with multiple small areas of radiolucency. Stability for 3 months	1	7.5	RUL lobectomy
3	E. C.	57M	Moderately advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily	4.5	Tomographic conformation interpreted as cavity; proved to be due to pleural changes following resorption of fluid and re-expansion of right lung	1.5	5.5	RLL lobectomy and decortication of lung
4	J. D. R.	44M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily†	7.5	No cavity by roentgenograms for 6 months or by tomography for 2 months	1.5	3.5	RUL and RML lobectomy
5	F. K.	34M	Minimal, fibrocaseous, without cavitation	Streptomycin (2× weekly) + PAS daily	8	Solid lesion (2 cm.) in lateral aspect, left mid-lung field; obliteration of left costophrenic angle	(1)‡	(1)‡	Wedge resection, anterior segment LUL

6	T. H.	35M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily§	9.5	Linear and nodular densities with "honeycombing" in shrunken RUL	(1.5)‡	(1.5)‡	Resection of apical and posterior segments RUL
7	G. G.	24M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily†	9	Stability on right for 4 months with cavitation	9	More than 3	Apical and posterior segmental resection RUL
8	R. C.	31M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily§	10	Bilateral stability for 6 months; a large coalescent infiltrate with small annular densities in shrunken RUL	4.5	11	RUL lobectomy
9	A. M.	34M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + isoniazid daily	11	1 to 2 months preoperatively small areas of breakdown within a dense lesion in LUL; multiple small solid lesions RUL; stability for 6 months	7.5	7.5	Resection of apico-posterior and anterior segments of LUL
10	J. McC.	41M	Far advanced, fibrocaseous, with cavitation	Isoniazid + PAS, —both daily	5	Stability of lesion on right, regressive resolution on left, decrease of size of LUL cavity; stability on left for 3 months	3	3	Apico-posterior segmental resection, LUL
11	A. J.	22M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily‖	11	Progressive clearing of extensive bilateral disease; development of marked fibrosis and shrinkage; persistence of cavity RUL	More than 5	More than 5	RUL lobectomy

TABLE 1—*Continued*

CASE NUMBER	PATIENT	AGE AND SEX	CLINICAL STATUS AT START OF PREOPERATIVE CHEMOTHERAPY	PREOPERATIVE CHEMOTHERAPY		STATUS AT TIME OF SURGERY BY ROENTGENOGRAPHY AND TOMOGRAPHY	PREOPERATIVE DURATION OF NONINFECTIOUSNESS		SURGICAL PROCEDURE
				Regimen	Duration		Microscopy	Culture	
					months		*months*	*months*	
12	C. D.	36M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily¶	10.5	Stability without definite evidence of cavity for 6 months	3.5	4	LUL lobectomy and segmental resection superior segment of LLL
13	W. A.	31M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + isoniazid daily¶	7.5	Progressive clearing of infiltrative lesions in RL and left lung field; stability for 3 months	6.5	6.5	RUL lobectomy, with additional thoracoplasty
14	L. R.	34M	Moderately advanced, fibrocaseous, with cavitation	Isoniazid + PAS, —both daily¶	5.5	No evidence of patent cavity	5.5	5.5	Resection of dorsal segment of LLL
15	L. B.	26M	Far advanced, fibrocaseous, with cavitation	Isoniazid + PAS, —both daily¶	6.5	Progressive shrinkage of lesion in RUL; clearing of seeding in left lung. Stability for 3 months	4.5	5.5	RUL lobectomy
16	M. T.	58F	Minimal, fibrocaseous, without cavitation	No chemotherapy	0	Questionable tuberculoma LUL,—2 cm.	0	0	Wedge resection
17	C. S.	28M	Moderately advanced, fibrocaseous, with cavitation	No chemotherapy	0	"Winking" cavity RUL 22 months' duration	3	3	Apical segmental lobectomy RUL

18	J. A.	25M	Far advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + PAS daily, for 4 months; then isoniazid added to the regimen (for 8 months)	12	Large cavity occupying most of superior segment, LLL	1.5	8.5	Segmental resection, superior segment LLL
19	W. B.	32M	Moderately advanced, fibrocaseous, with cavitation	Streptomycin (2× weekly) + isoniazid, initiated 17 months after 90 days of streptomycin (2× weekly) + PAS daily	2	Patent cavity LUL	0	0	Segmental resection, apico-posterior segment LUL

* 4 months (streptomycin daily plus PAS,—2 previous weeks).

† 3.5 months (streptomycin daily plus PAS,—4 to 5.5 previous months).

‡ Single isolated specimen showed growth of tubercle bacilli after 5 to 7 months of noninfectiousness.

§ 6–6.5 months (isoniazid—3.5 previous months).

‖ 10 months (streptomycin 3 times weekly with daily PAS,—1 month previously).

¶ All patients had received previous courses of streptomycin (2 times weekly or daily) plus PAS daily, for 1 to 6 months, terminating 3 to 21 months prior to start of the preoperative course of chemotherapy.

patients were "re-treatment" cases, having had previous courses of antimicrobial therapy terminating three to twenty-one months prior to institution of the pre-operative course of chemotherapy. The remaining 4 patients were "control" cases, 2 of whom had received either no, or essentially no, chemotherapy pre-operatively, while 2 had patent open cavities at the time of resection.

The details of each patient's illness with respect to duration of chemotherapy, drug regimen used, and status of infectiousness at the time of surgery are described in table 1. In addition, detailed case reports and a discussion of the clinico-pathologic observations made in relation to this study are presented in a recent publication by Auerbach and his associates (27).

Microbiologic Technique

Four basic differences exist between the microbiologic technique utilized herein and the techniques which have been described previously.

1. It is a well-known fact that tubercle bacilli may be maintained in a viable state for long periods of time when frozen. Thus, all specimens were *frozen immediately after surgery* and were not thawed until immediately prior to dissection and cultivation of the lesions.
2. As saline as well as distilled water are known to be deleterious to many bacteria, the tissue was blended and homogenized in a *balanced liquid medium* containing a sufficient quantity of albumin to permit neutralization of certain of the toxic components of the necrotic material.
3. In order to facilitate neutralization of the toxic components of the necrotic material, the homogenate was centrifuged and the sedimented fraction *washed three times with fresh quantities of the albumin-containing medium.*
4. All cultures were *incubated for nine months* at 37°C. in a medium which *does not deteriorate* under such conditions.

The method utilized throughout this study may be described in detail as follows:

Each specimen was removed aseptically at the time of surgery, immediately frozen in a deep-freeze refrigerator in a sterile closed glass container, and subsequently thawed at 5°C. during a period of twelve to fifteen hours immediately prior to dissection.

The lesions were dissected by the pathologist in the presence of the clinician and bacteriologist, care being taken not to contaminate one lesion with material from another. Specimens were handled at this stage and during all subsequent cultural operations with sterile aseptic "mask and gown" technique in a closed sterile area.[5] Separate sterile gloves

[5] A closed sterile area under a negative pressure of approximately one-fourth inch of water. The area is supplied with sterile air by means of a double bank of disposable Air Mat Dry Filters (Type K, manufactured by the Airgard Manufacturing Co., Chicago, Illinois). Negative pressure is maintained by the use of a blower and the exhaust air is passed over another double bank of the same type of filters described above. The area is covered by two types of ultraviolet: Hi Intensity Ultraviolet for sterilization of the area and Standard Ultraviolet for use while personnel are operating within the area. Access to the area is gained by the use of an air lock which is similarly covered by both types of ultraviolet lighting. During the actual handling of specimens or microbiologic cultures, *all* ultraviolet lights have been turned off in order to avoid possible death of tubercle bacilli which might be exposed directly to the rays.

were used for each specimen, and separate sterile instruments were used to dissect each lesion from each specimen.

Each lesion was weighed, macerated with sterile scissors, and a direct smear was made by mashing a piece of tissue between two slides. The tissue was then suspended in a sufficient volume of albumin medium to give a tissue concentration ranging generally from 1 to 5 per cent.

The medium used throughout the study for all dilutions, washings, and cultures was the Dubos liquid medium containing 0.5 per cent albumin and 25 units of penicillin per ml.; Tween® 80 was not included in the medium except when specifically stated.

The tissue was blended in a Waring Blendor, homogenized in a sterile glass Porter Mill[6, 7] until a uniform suspension was obtained, and the suspension was then centrifuged for thirty minutes in an angle centrifuge (1,750 r.p.m.). The sediment was washed three times with albumin broth and the residual sediment and supernatant fractions were recovered. The first three supernatant fractions were pooled, brought to an average volume of 50 ml. by addition of albumin broth, and incubated; the fourth supernatant was incubated separately. The sediment was brought to a final volume of 1.1 ml. by addition of albumin broth[8] and the resultant suspension was subdivided as follows: 0.1 ml. of suspension was inoculated into each of 3 screw-capped tubes (20 x 125 or 150 mm. in size) containing approximately 3.5 ml. of 0.5 per cent albumin medium, 0.1 ml. into each of 3 screw-capped tubes containing approximately 3.5 ml. of this same medium to which had been added 0.02 per cent Tween® 80, and 0.1 ml. was inoculated subcutaneously into each of 3 guinea pigs.

Length of incubation and frequency of subculture on solid medium: All cultures were incubated for a period of eight to nine months unless growth of *M. tuberculosis* from a particular lesion was demonstrated at an earlier date. Although this long incubation period was used from the beginning of the study, the frequency with which viable tubercle bacilli would be obtained from the liquid cultures after more than two or three months of incubation was not appreciated at first, and frequent observations were not made after the ninth to twelfth weeks of incubation. Indeed, in order to avoid contamination, culture flasks and tubes were opened only after approximately five to six, eight to twelve, and thirty to forty weeks of incubation. At such times, however, 0.1 ml. was transferred from each tube to the surface of an oleic-acid-albumin agar plate which was then incubated for twenty-one days at 37°C. and subsequently examined for colonial growth. Fresh sterile broth, comparable to that originally used for each culture tube, was added if necessary at these time intervals in order to bring back to volume.

Animal inoculations: Guinea pigs were tuberculin tested at approximately six weeks and at three and six months after injection; those showing positive tuberculin reactions at six months or earlier were sacrificed at the end of the sixth month for histopathologic study. Animals which showed negative reactions at this time period were held for study at the end of the ninth month.

In no instance was there evidence of tuberculous infection in guinea pigs infected with tissue from lesions which failed to show viable tubercle bacilli on culture. Demonstration of morphologically typical colonies of *Mycobacterium tuberculosis* by the procedure described above was accepted, therefore, as evidence of viable tubercle bacilli within the lesion in question.

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[6] Manufactured by Macalaster & Bicknell, Cambridge, Massachusetts.

[7] In all instances homogenization was carried out in a partially closed sterile hood located within the sterile area previously described. The hood is equipped with two types of ultraviolet (Hi Intensity and Standard), the light radiating down from the top of the hood and up through 1-inch circular holes in the floor of the hood. These ultraviolet lights are used for sterilization of the area just prior to, and immediately after, homogenization.

[8] In occasional instances, the size of the specimen made it necessary to increase the volume of broth used. In such instances, the number of culture tubes and/or guinea pigs inoculated was such that the entire volume of suspension was utilized.

Drug-susceptibility tests: All cultures of *M. tuberculosis* recovered from the resected specimens were subcultured and their susceptibility to streptomycin and isoniazid was determined by the conventional procedure in a liquid Tween®-albumin medium.

Pathogenicity for guinea pigs of the bacilli isolated on culture: In addition, the ability of the bacilli obtained on culture to produce tuberculous disease was established by reinoculation of 1 to 10 million viable cell units (average, 4.6 million)[9] into guinea pigs by the intravenous route. The standard for comparison consisted of comparable numbers of viable cell units of the H37Rv strain of *M. tuberculosis* injected simultaneously into comparable animals. Four guinea pigs (male, 250 gm.) were used for each culture. The virulence of the infecting strain was determined by the extent of tuberculous disease observed histopathologically at the time of death or sacrifice and by the rate at which the disease brought about death of the infected animals.

RESULTS

Microbiologic Observations

As mentioned previously, 31 necrotic lesions from 19 patients are described herein. The details pertaining to each patient's illness are summarized in tables 1 and 2. The clinicopathologic observations made in relation to the present microbiologic study are discussed in a communication by Auerbach and his associates (27), while brief comment concerning a portion of the microbiologic findings has been made recently by Hobby and associates (1).

The nature of the necrotic tuberculous lesions studied[10] and the microscopic and cultural observations made thereon are presented below.

Lesion 1, Case 1 (J. R.): A single 2 mm. necrotic focus was removed in its entirety for culture. This specimen was negative for acid-fast bacilli by microscopy. Guinea pigs inoculated with an aliquot of the specimen, according to the method described, remained free of tuberculosis. Long-term incubation of another aliquot by the cultural methods described likewise revealed no viable tubercle bacilli.

Lesion 2, Case 2 (E. H.): Part of a large inspissated cavity was removed for microbiologic studies. Small numbers of acid-fast bacilli were observed on microscopy. Guinea pigs inoculated with aliquots of the specimen showed extensive tuberculous disease. Cultures made from another aliquot of the specimen, according to the procedure described above, revealed the presence of *M. tuberculosis* in all culture tubes after an incubation period of only 35 days.

Lesion 3, Case 2 (E. H.): A filled-in cavity with necrotic foci showed no acid-fast bacilli by microscopy. Nevertheless, guinea pigs inoculated with a portion of the specimen developed extensive tuberculosis. Moreover, cultivation of the remaining portion of the specimen revealed viable tubercle bacilli in all culture tubes on incubation for 35 days.

Lesion 4, Case 3 (E. C.): A specimen containing hard necrotic foci showed moderate numbers of acid-fast bacilli by microscopy. Although guinea pig inoculation of a portion of this specimen failed to produce tuberculous infection, cultivation of the

[9] The number of viable cell units was determined by the conventional pour-plate technique, using the oleic-acid-albumin solid medium and an incubation period of twenty-one days. Previous experience has indicated that counts made by this procedure are comparable to those obtained by the method of Fenner and associates (28).

[10] The case numbers used herein coincide with those used by Auerbach and associates (27).

remaining portion of the specimen revealed viable tubercle bacilli after prolonged incubation. No growth was detected in the subcultures made onto solid medium after 63 days' incubation but viable tubercle bacilli were isolated from one of 8 culture tubes after 146 days.

Lesion 5. Case 3 (*E. C.*): The soft liquefied necrotic material from an inspissated cavity showed 1 to 2 acid-fast bacilli per microscopic field, but revealed no viable tubercle bacilli either by guinea pig inoculation of an aliquot of the specimen or by prolonged incubation of the residual portion of the specimen for a period of 284 days. In contrast, tubercle bacilli were not detected in the wall of this cavity by microscopy or by inoculation of the specimen into guinea pigs. Cultivation of a portion of the cavity wall, however, revealed the presence of viable tubercle bacilli in one of 6 liquid culture tubes. The presence of the bacilli was demonstrable after an incubation period of 63 days, although no growth on culture was obtained at 45 days.

Lesion 6. Case 4 (*J. D. R.*): A portion of the necrotic contents of an inspissated cavity in the right upper lobe showed no acid-fast bacilli by microscopy, by guinea pig inoculation of a portion of the specimen, or by prolonged incubation (284 days) of the residual portion of the specimen.

Lesion 7. Case 4 (*J. D. R.*): Putty-like foci at the base of the same lobe likewise showed no viable tubercle bacilli by guinea pig inoculation or prolonged cultivation (284 days) of portions of the specimen. A few sparsely scattered acid-fast bacilli were observed, however, by microscopy.

Lesion 8. Case 5 (*F. K.*): Half of an inspissated cavity, including a portion of the wall and of the liquefied contents, was removed for microbiologic study. Innumerable acid-fast bacilli were visible by microscopy. Guinea pig inoculation of a portion of the specimen, however, as well as prolonged cultivation for a period of 284 days failed to reveal *M. tuberculosis* in culturable form.

Lesion 9. Case 6 (*T. H.*): A specimen containing chalky foci showed no evidence of acid-fast bacilli by microscopy. Guinea pig inoculation of a portion of the specimen failed to produce tuberculous disease, and prolonged incubation of the residual portion of the lesion for a period of 284 days also failed to reveal the presence of tubercle bacilli.

Lesion 10. Case 7 (*G. G.*): A single specimen consisting of pus from an inspissated cavity and several small necrotic foci revealed no tubercle bacilli by microscopy. Guinea pigs inoculated with a portion of the lesion developed extensive tuberculosis, however, while viable tubercle bacilli were demonstrated in liquid cultures of the residual portion of the lesion after 62 days' incubation. Viable bacilli were present in all of the liquid culture tubes.

Lesion 11. Case 8 (*R. C.*): Acid-fast bacilli were observed by microscopy of the necrotic contents of a right upper lobe inspissated cavity. Guinea pigs inoculated with a portion of this specimen failed to develop tuberculosis. Cultivation of the remaining portion of the lesion revealed growth of tubercle bacilli in subcultures made from one of 40 culture tubes after an incubation period of 48 days.

Lesion 12. Case 8 (*R. C.*): A portion of the unsloughed satellite necrotic lesions showed acid-fast bacilli by microscopy. Guinea pig inoculation of a portion of this specimen failed to reveal growth of tubercle bacilli *in vivo*, although cultivation of the residual portion of the lesion *in vitro* revealed viable tubercle bacilli in one of 9 culture tubes within 36 days.

Lesion 13. Case 9 (*A. M.*): Microscopic examination revealed the presence of small numbers of acid-fast bacilli, while viable tubercle bacilli were demonstrated by guinea

TABLE 2

CORRELATION OF MICROBIOLOGIC AND PATHOLOGIC OBSERVATIONS OF RESECTED TUBERCULOUS LESIONS

CASE NUMBER	NAME	ROENTGENOGRAPHIC OBSERVATIONS OF INFLATED RESECTED SPECIMEN AND PATHOLOGIC OBSERVATIONS	LESIONS SELECTED FOR STUDY		MICROBIOLOGIC OBSERVATIONS ON LESION		
			Number	Pathologic Description	Acid-Fast Bacilli Detected by Microscopy	Growth of *M. tuberculosis* Detected by:	
						Guinea Pig Inoculation	Prolonged Cultivation *in Vitro*
1	J. R.	Only a 3 mm. yellow necrotic focus	1	A single 2 mm. necrotic focus (entire focus taken for culture)	−	−	−
2	E. H.	Necrotic foci and filled-in cavities measuring 3 mm. to 3 cm. in size; the bronchocavitary junction was patent and re-epithelized	2 3	Part of large inspissated cavity Filled-in cavity with necrotic foci	+ −	+ +	+ +
3	E. C.	Small inspissated cavity in superior segment of RLL, not corresponding to lucency seen on tomograms, with adjacent 2 to 5 mm. necrotic foci	4 5	Hard necrotic foci Soft liquefied necrotic contents of an inspissated cavity Cavity wall	+ + −	− − −	+ − +
4	J. D. R.	No open cavity; an inspissated cavity RUL, with numerous small, chalky and putty-like foci	6 7	A portion of the necrotic contents of inspissated cavity, RUL Putty-like foci at base of same lobe	− +	− −	− −
5	F. K.	Only a small dense nodule which on sectioning proved to be a 2 cm. cavity with liquefied contents	8	Half of the inspissated cavity, including a portion of the wall and of the liquefied contents	+	−	−
6	T. H.	Multiple 1 to 2 mm. chalky foci with areas of fibrosis; no cavity	9	Chalky foci	−	−	−

7	G. G.	Multiple small closed lesions consisting on section of an inspissated cavity and 3 to 5 mm. unsloughed necrotic foci	10	Pus from the inspissated cavity and several small necrotic foci	−	+	+
8	R. C.	Inspissated cavity measuring 3 x 4.5 x 2.5 cm., with a patent re-epithelized communicating bronchus and adjacent 1 to 2 mm. necrotic foci	11	Necrotic contents of the RUL inspissated cavity	+	−	+
			12	A portion of the unsloughed satellite necrotic lesions	+	−	+
9	A. M.	Multiple solid lesions without a patent cavity; small inspissated cavities and multiple 3 to 6 mm. stone-hard (calcific) foci	13	Portions of the small inspissated cavities	+	+	+
10	J. McC.	Only inspissated cavities measuring 7 to 9 mm. and necrotic foci measuring 5 to 12 mm.	14	Two pooled cavities, including the capsule and liquefied contents of each	+	+	+
			15	Cavity wall and liquefied contents of another cavity in apical portion of the LUL	−	+	+
			16	Two small necrotic foci, one of which was soft and putty-like	−	−	+
11	A. J.	A widely patent cavity measuring 3.5 x 2 x 3 cm., with smooth lining to which 1 minute yellow caseous fragment was attached; patent communicating bronchi opening into the cavity were demonstrated	17	Wall and tissue surrounding a clean-walled cavity	+	−	+
			18	Clean-walled cavity lying just inferior to and communicating with the above cavity (#17), and with small necrotic zone	−	−	+
			19	Two 4 mm. putty-like foci	+	−	+
			20	Clump of small firm yellow foci	+	−	+

TABLE 2—*Continued*

CASE NUMBER	NAME	ROENTGENOGRAPHIC OBSERVATIONS OF INFLATED RESECTED SPECIMEN AND PATHOLOGIC OBSERVATIONS	LESIONS SELECTED FOR STUDY		MICROBIOLOGIC OBSERVATIONS ON LESION		
			Number	Pathologic Description	Acid-Fast Bacilli Detected by Microscopy	Growth of *M. tuberculosis* Detected by:	
						Guinea Pig Inoculation	Prolonged Cultivation *in Vitro*
12	C. D.	Multiple inspissated cavities measuring up to 2 cm., and many small encapsulated caseous foci	21	Central necrotic portion of a 5 mm. inspissated cavity	+	−	+
				Cavity wall	−	−	+
			22	Wall and central necrotic component of a second inspissated cavity	+	−	+
			23	Two small (pooled) necrotic foci	+	−	+
13	W. A.	Gross examination revealed occasional necrotic foci (1 to 3 mm.) with no evidence of presence of an inspissated cavity. Serial sections revealed a small cavity space, lined by a zone of hyalinized connective tissue, but with no specific tuberculous elements present within it	24	Half of a clean-walled cavity, including the wall and lung parenchyma	−	−	+
14	L. R.	Inspissated cavity (1.5 x 1 cm.) with liquefied contents; a patent communicating bronchus was demonstrated. Multiple adjacent 2.5 mm. necrotic foci	25	Central necrotic component of an inspissated cavity	+	−	+
				Wall of cavity	−	−	−
15	L. B.	Small compressed lobe with a 4 mm. inspissated cavity in its superior portion and a radial scar (3.5 x 1.0 cm. x 7 mm.) in its posterior portion	26	Lung tissue containing small liquefied areas	+	−	−
			27	Bunch of small necrotic partially calcified foci	−	−	+

16	M. T.	Necrotic focus (never sloughed) surrounded by tuberculous granulation tissue	28	5 x 7 mm. lesion,—minimal but entirely necrotic	+	+	+
17	C. S.	A 6 mm. inspissated cavity filled with soft yellow material	29	Cavity wall and much of the soft necrotic material from an inspissated cavity	+	+	+
18	J. A.	A cavity 4 cm. x 2.5 cm., with an open bronchus	30	Cavity wall, surrounding tissue, and some necrotic material	+	+	+
19	W. B.	A 1.5 cm. cavity lined by brown granular surface, with a draining bronchus	31	A single necrotic focus	−	+	+

+ Indicates the demonstration of acid-fast bacilli on direct microscopic examination of macerated tissue; the demonstration of growth of *M. tuberculosis* by development of progressive and extensive tuberculous disease in guinea pigs; *or* the demonstration of growth of *M. tuberculosis* by recovery of viable strains on *in vitro* cultivation by the methods described herein.

− Indicates the failure to demonstrate acid-fast bacilli and/or growth of *M. tuberculosis*, as evidenced by the above criteria.

pig inoculation and by cultivation, according to the methods described, in portions of small inspissated cavities. Growth was apparent in subcultures made from all culture tubes after 62 days.

Lesions 14–16. Case 10 (*J. McC.*): Viable tubercle bacilli were recovered on culture after an incubation period of 41 days from (*a*) two pooled cavities including the capsule and liquefied contents of each (lesion 14), (*b*) the cavity wall and liquefied contents of another cavity in the apical portion of the left upper lobe (lesion 15) and, (*c*) from two small necrotic foci, one of which was soft and putty-like (lesion 16). The presence of viable tubercle bacilli was detected in all culture tubes of all specimens. Direct microscopic examination revealed acid-fast bacilli in the first of these three specimens only, while guinea pigs inoculated with portions of the first two specimens but not the third specimen developed tuberculosis.

Lesions 17–20. Case 11 (*A. J.*): The wall and tissue surrounding a clean-walled cavity (lesion 17) revealed acid-fast bacilli by microscopy. A clean-walled cavity (lesion 18) lying just inferior to, and communicating with, the previously mentioned cavity and with a small necrotic zone, in contrast, showed no acid-fast bacilli on microscopic examination. Two 4 mm. putty-like foci (lesion 19) revealed acid-fast bacilli on microscopy, as did a clump of small firm yellow foci (lesion 20). All four specimens failed to produce tuberculosis in guinea pigs when aliquots of each were injected according to the method described. On cultivation of the residual portions of these four specimens, growth of *M. tuberculosis* was detected in subcultures from 2 of 8 culture tubes inoculated with lesion 17, from 4 of 7 culture tubes inoculated with lesion 18, from 4 of 8 culture tubes inoculated with lesion 19, and from one of 10 culture tubes inoculated with lesion 20. Whereas viable tubercle bacilli were detected in one culture tube on lesion 17 within 35 days, in all other instances they were not apparent at 83 days but were apparent after an incubation period of 155 days.

Lesion 21. Case 12 (*C. D.*): The central necrotic portion of a 5 mm. inspissated cavity showed acid-fast bacilli on microscopy; the wall of this cavity failed to show such organisms on microscopic examination. Both the central necrotic portion of this cavity and the cavity wall failed to produce tuberculosis in guinea pigs when inoculated according to the method described. Both portions of the cavity showed *M. tuberculosis* when cultivated for prolonged periods of time. Two of 8 culture tubes inoculated with an aliquot of the central necrotic portion and 3 of 8 tubes inoculated with an aliquot of the cavity wall showed growth on subculture at 146 days, although not at 63 days.

Lesion 22. Case 12 (*C. D.*): The wall and central necrotic component of a second inspissated cavity were pooled for bacteriologic study. Again, acid-fast bacilli were detected by microscopy. Although an aliquot of the specimen failed to produce tuberculosis when inoculated into guinea pigs, the presence of *M. tuberculosis* was demonstrated in 2 of 8 culture tubes inoculated with the residual portion of the lesion. Growth was apparent on subcultures made at 146 days, although not at 63 days.

Lesion 23. Case 12 (*C. D.*): Two small (pooled) necrotic foci showed acid-fast bacilli on microscopy. Although guinea pigs inoculated with a portion of the specimen failed to develop tuberculosis, viable tubercle bacilli were apparent in 3 of 8 culture tubes inoculated with the residual portion of the lesion. Growth was apparent on subcultures made at 146 days, but not at 63 days.

Lesion 24. Case 13 (*W. A.*): Half of a clean-walled cavity, including the wall and lung parenchyma, was selected for study. Microscopy revealed acid-fast bacilli in this specimen. Guinea pigs inoculated with a portion of it failed to develop tubercu-

losis. Cultivation of the remaining portion of the lesion revealed viable tubercle bacilli in 2 of 8 culture tubes after prolonged incubation. Growth was detected in subcultures made at 274 days, but not at 223 days.

Lesion 25. Case 14 (*L. R.*): The central necrotic component of an inspissated cavity and the wall of this cavity were studied separately. Acid-fast bacilli were detected on microscopy in the central necrotic component but not in the cavity wall. Neither produced tuberculosis in guinea pigs when inoculated by the method described. Cultivation of the cavity wall for a period of 284 days revealed no viable tubercle bacilli. A viable strain of *M. tuberculosis* was detected, however, in one of 8 culture tubes inoculated with the central necrotic portion of the lesion after prolonged incubation. Growth was apparent in subcultures made at 146, but not at 63 days.

Lesion 26. Case 15 (*L. B.*): A piece of lung tissue containing small liquefied areas was snipped for bacteriologic study. Although acid-fast bacilli were detected in this specimen by microscopy, growth of *M. tuberculosis* was not detected by inoculation of portions of the specimen into guinea pigs or by prolonged cultivation (265 days) of the remainder of the specimen.

Lesion 27. Case 15 (*L. B.*): A bunch of small necrotic, partially calcified foci showed no evidence of acid-fast bacilli by microscopy, but did show the presence of *M. tuberculosis* on cultivation of a portion of the specimen for only 35 days. Growth was observed in subcultures from 2 of 7 culture tubes at this time period. Guinea pigs inoculated with the residual portion of the lesion failed to develop tuberculosis.

Lesion 28. Case 16 (*M. T.*): A 5- by 7- mm. minimal but entirely necrotic lesion was removed for study. Resection had been performed due to question of carcinoma. On microscopy, a few sparsely scattered bundles of acid-fast bacilli were detected in the lesion. Inoculation of a portion of the lesion into guinea pigs produced extensive tuberculosis, while the presence of *M. tuberculosis* was detected within 35 days in all culture tubes inoculated with the residual portion of the lesion.

Lesion 29. Case 17 (*C. S.*): The cavity wall and much of the soft necrotic material from an inspissated cavity were pooled for microbiologic study. On microscopy, acid-fast bacilli were detected in the specimen. Inoculation of a portion of the lesion into guinea pigs produced extensive disease, while cultivation of the remainder revealed *M. tuberculosis* in all culture tubes within 49 days.

Lesion 30. Case 18 (*J. A.*): A cavity wall with surrounding tissue and some necrotic material was removed for study. Microscopy revealed acid-fast bacilli, while inoculation of guinea pigs with a portion of the specimen produced extensive tuberculosis in these animals. Cultivation of the remaining portion of the lesion revealed viable tubercle bacilli in all culture tubes after 62 days' incubation.

Lesion 31. Case 19 (*W. B.*): A single necrotic focus was removed from a specimen in which no cavities were found and in which it was indeed difficult to detect any lesions whatsoever. No acid-fast bacilli were detected on microscopy. Inoculation of a portion of the tissue into guinea pigs produced extensive tuberculosis, however, and cultivation of the remainder of the lesion showed growth of *M. tuberculosis* in subcultures made from all culture tubes after an incubation period of 62 days.

As illustrated in table 2 and as indicated also by the data presented above, viable tubercle bacilli were recovered from 25 of 31 lesions, or from 15 of 19 patients. In contrast, acid-fast bacilli were detected on microscopy in only 21 of the 31 lesions. It is of interest that only 3 lesions showed acid-fast bacilli on microscopic examination of the specimen which could not be cultivated by

TABLE 3

THE FREQUENCY AND RATE OF APPEARANCE OF GROWTH OF MYCOBACTERIUM TUBERCULOSIS IN CULTURES OF RESECTED PULMONARY LESIONS

LESION NUMBER*	MICROBIOLOGIC OBSERVATIONS				
	Acid-Fast Bacilli on Microscopy	Growth of *M. tuberculosis*			
		Number of Culture Tubes† Showing Viable Cells	Total Number of Culture Tubes† Used	Time (Days) at Which Viable Cells Were Detected	
2	Present	17	17		<35
3	Absent	2	2		<35
5	Present	1	13	>45	<63
10	Absent	7	7		<62
11	Present	1	40	>36	<48
12	Present	1	9		<36
13	Present	8	8		<62
14	Present	8	8		<41
15	Absent	8	8		<41
16	Absent	5	8		<41
17	Present	2	8		<35‡
27	Absent	2	7		<35
28	Present	9	9		<35
29	Present	8	8		<49
30	Present	10	10		<62
31	Present	8	8		<62
4	Present	1	8	>63	<146
18	Absent	4	7	>83	<155
19	Present	4	8	>83	<155
20	Present	1	10		<83
21	Present	5	16	>63	<146
22	Present	2	8	>63	<146
23	Present	3	8	>63	<146
24	Present	2	8	>223	<274
25	Present	1	16	>63	<146

* The lesion numbers used in this table coincide with those used in the text of the manuscript and in tables 1 and 2.

† Whereas the term "culture tube" has been used throughout this table and throughout the section of the manuscript describing the patients from whom these lesions were obtained, nonetheless, as indicated in the section on Materials and Methods, culture flasks (rather than tubes) were used in some instances. The term "culture tube" is used herein to refer to tubes and/or flasks.

‡ Growth was observed in subcultures made from one tube at 35 days, but from a second tube only after an incubation of more than 83 but less than 155 days.

the cultural methods described. In contrast, growth of *M. tuberculosis* was observed in cultures of 7 lesions in which acid-fast bacilli had not been detected by microscopy.

Of the 25 lesions from which strains of *M. tuberculosis* were isolated by the culture methods used, 9 showed evidence of such bacilli only after more than 63 days. Of these 9, one required only 83 days' incubation in liquid medium, 2 required more than 83 days, and one required more than 223 days; the re-

maining 5 required between 63 and 146 or 155 days. Material from a single lesion, although yielding viable tubercle bacilli in a single culture tube incubated 35 days, required more than 83 days' incubation before viable bacilli could be demonstrated in a second culture tube. Whereas viable cells were detected in *all* culture tubes inoculated with 10 of the 16 lesions which showed viable tubercle bacilli after an incubation period of 63 days or less, growth was observed on subcultures from only a small fraction of the tubes[11] inoculated with each of the 9 lesions requiring more prolonged incubation.[12] Eight of the 9 lesions in which viable tubercle bacilli were detected only after prolonged periods of time had shown acid-fast bacilli on direct microscopic examination of the lesion; only 11 of the 16 lesions which showed viable tubercle bacilli after shorter periods of time had shown acid-fast bacilli on direct microscopy (table 3).

Infectivity of resected tissue for guinea pigs: Only 10 of the 25 lesions which showed growth of tubercle bacilli by the cultural methods used were capable of producing tuberculosis on direct inoculation into guinea pigs. These 10 coincide completely with the 10 lesions mentioned above, which showed viable cells in *all* tubes inoculated, within an incubation period of 63 days. Thus, it appears that only those lesions which contained organisms capable of multiplying readily *in vitro* under the conditions used were capable of producing tuberculosis in guinea pigs on direct inoculation. Since 3 of these 10 lesions showed no acid-fast bacilli on direct microscopy[13], the ability of the organisms to grow in culture and to produce disease in animals may be related more to the metabolic state of the microorganisms than to the actual numbers present.

Infectivity of subcultured microorganisms for guinea pigs: It should be emphasized, however, that, on subculture, all strains of tubercle bacilli recovered in the present study which have been tested to date have been observed to be highly pathogenic for guinea pigs. In like manner all are highly susceptible to 0.1 γ or less of isoniazid per ml. of medium, while only one of the strains recovered from these lesions requires more than 1 γ of streptomycin per ml. of medium for inhibition of growth.

Analysis of Microbiologic Observations in Relation to Clinico-pathologic Factors

As may be seen from the foregoing data and from tables 1 and 2, viable tubercle bacilli were recovered from 7 of 11 "original treatment" cases, or from 15 of the 20 lesions from them. Likewise, viable strains of *M. tuberculosis* were recovered

[11] Attention is called to the fact that growth of *M. tuberculosis* might well have been observed in subcultures from a higher percentage of culture tubes if all had been incubated for a longer period of time. Cultures of all lesions failing to show the presence of *M. tuberculosis* were observed for a period of nine months. However, culture tubes on lesions showing the presence of this organism were held only until definite evidence was available to prove that the lesion had contained viable tubercle bacilli which could be cultivated *in vitro* (table 3).

[12] Whereas viable tubercle bacilli were recovered in some instances when cultivated in either the albumin or Tween®-albumin medium, such organisms frequently were recovered in the albumin medium when they failed to grow in the presence of Tween® 80.

[13] All stained preparations were examined for a period of not less than twenty minutes. In essentially all instances, preparations showing no acid-fast bacilli were examined for at least forty minutes before it was concluded that no acid-fast bacilli were present.

from 4 of the 4 "re-treatment" cases, or from 6 of the 6 lesions from such cases, and from all 4 lesions from 4 "control" patients. Growth of *M. tuberculosis* was demonstrated frequently by the method used in this study from "original" and from "re-treatment" cases; it was demonstrated as frequently from patients who had had eight to twelve months as from those who had had only four to eight months of preoperative chemotherapy. In like manner, the frequency with which viable tubercle bacilli were detected in the resected lesions of patients who had been noninfectious for as long as three to twelve months preoperatively was as high as the frequency with which strains of *M. tuberculosis* were cultivated from lesions resected after shorter periods of noninfectiousness.

The majority of patients from whom lesions were obtained for this study had received streptomycin twice weekly in combination with para-aminosalicylic acid daily. None had received daily streptomycin during the preoperative period, while only 5 had received daily isoniazid preoperatively. *Mycobacterium tuberculosis* was cultivated from 14 of 19 lesions from patients who received twice weekly streptomycin with daily para-aminosalicylic acid, or from 6 of 10 patients. Strains of *M. tuberculosis* were grown also from 7 of 8 lesions removed from 5 patients who had received isoniazid daily (in conjunction with para-aminosalicylic acid daily or twice-weekly streptomycin) during the preoperative period, or from all 5 of these patients.

Analysis of the pathologic observations on the "closed" or healed lesions discussed in this report has indicated that growth of *M. tuberculosis* was demonstrated on cultivation of tissue by the method described herein from 12 of 17 inspissated cavities, from a single open cavity with morphologic evidence of healing, and from 8 of 9 nonliquefied necrotic lesions. In 3 instances, the wall and central necrotic component of an inspissated cavity were cultured separately; viable tubercle bacilli were recovered from the wall of the first one, from the central necrotic zone of the second one, and from both portions of the third such cavity. Based on these data, it does not appear possible to correlate the presence of viable tubercle bacilli with any specific type of "closed" or healed lesion, nor specifically with either portion of an inspissated cavity.

Although the number of lesions cultured in the present study from "control" patients who either had had no, or essentially no, chemotherapy preoperatively or had patent open cavities at the time of resection was small, nonetheless, growth of *M. tuberculosis* was readily demonstrated in all instances, e.g., from 4 lesions from 4 patients. This is in agreement with the observations of D'Esopo and his associates (2, 3), who have observed that viable tubercle bacilli may be demonstrated readily by conventional techniques in lesions from such patients.

It is of special interest that 15 of the 16 lesions which yielded *Mycobacterium tuberculosis* after an incubation period of 63 days or less were from "original treatment" cases or "control" cases. Only one (Case 15, L.B.) was from a "re-treatment" patient who had had 6.5 months of preoperative chemotherapy (isoniazid in conjunction with para-aminosalicylic acid) and had been noninfectious for a period of 4.5 (on microscopy) to 5.5 (on culture) months preoperatively.

In contrast, 5 of 9 lesions, cultures of which required an incubation period of more than 63 days for demonstration of viable cells, were ones from "retreatment" cases. One of the remaining 4 was from a patient (Case 3, E. C.) who had received 4.5 months of streptomycin and para-aminosalicylic acid preoperatively and had been noninfectious for a period of 5.5 months prior to resection. The remaining 3 were from a single patient (Case 11, A. J.) who had received 11 months of streptomycin and para-aminosalicylic acid preoperatively and had been noninfectious for a period of more than 5 months prior to surgery.

DISCUSSION

The significance of the microbiologic results reported herein is dependent in part upon the nature of the lesions studied. It has been recognized throughout that tubercle bacilli from open cavities generally may be cultivated *in vitro* with little or no difficulty. The observations of D'Esopo and his associates (3) have been confined to those residual lesions resected from patients meeting "target point" criteria, as defined by them, namely, closure of all cavities, a consistently noninfectious status for "several" months, and roentgenographic stability. The importance of cavity closure has been emphasized by D'Esopo, who has stated that "the only real correlation between nonviability and the lesions is closure of the cavities," and that morphologic evidence must take precedence over roentgenographic evidence of cavity closure (6).

It became apparent early in the course of the present study that, while the definition of "target point" lesions might appear accurate and concise, in practice it was vague and indefinite. Tomographic interpretations of "extensive honey-combing" or of "probable multiple small excavations" proved frequently, on thorough examination of the resected specimens, to consist of areas of pulmonary fibrosis and small encapsulated caseous or calcific foci. Furthermore, the significance of continued infectiousness, as evidenced by microscopy, after cultures had ceased to yield tubercle bacilli is not taken into consideration in stating the criteria for "target point" lesions. In addition, no evidence is available to indicate how long a patient must be noninfectious during the preoperative period in order to comply with "target point" criteria.

For the purpose of the present study, cavity closure was determined by evaluation not only of preoperative laminagrams but also of roentgenograms of the inflated freshly resected specimen and by careful morphologic examination, and cavity closure or healing served as the primary basis for differentiation of lesions.

Microbiologic observations on tuberculous lesions from 19 patients have been described in the present report. Eleven of these 19 patients were "original treatment" cases, 5 of whom met all "target point" criteria as defined by D'Esopo and associates (3). Three of these 11 patients met "target point" criteria with the exception that microscopy, although not the repeated cultures obtained, suggested an infectious status until one to two months prior to resection. Two of the remaining 3 patients in the group met the criteria for "target point" lesions in all respects except that each showed evidence of infectiousness, by virtue of

the demonstration of growth of tubercle bacilli in one isolated specimen four to six weeks prior to resection, after having been noninfectious for a period of several months previously. The last patient in the series had had eleven months of preoperative chemotherapy and had been noninfectious, as evidenced by microscopy and also by cultural studies, for more than five months preoperatively. In this patient only one small necrotic focus was attached to the inner cavity wall, which otherwise contained no specific evidence of the tuberculous process; peripheral to the cavity wall were small encapsulated necrotic foci.

Four of the 19 patients studied were "re-treatment" patients who had had previous courses of antimicrobial therapy terminating three to twenty-one months prior to institution of the preoperative course of chemotherapy. All 4 of these patients had received five to ten months or more of chemotherapy immediately preoperatively. All were noninfectious for at least three to six months preoperatively.

Four of the 19 patients included in the study were "control" cases. Of these, 2 had received either no, or essentially no, chemotherapy preoperatively, while 2 had patent open cavities at the time of resection. One of the 2 patients with open cavities had had twelve months of preoperative chemotherapy and had been noninfectious, as evidenced by cultural studies (although not by microscopy) for eight and one-half months prior to surgery.

Twenty-six of the 31 lesions from these 19 patients were classified as "closed" necrotic lesions on the basis of morphology. As previously indicated, one was classified as an open lesion with morphologic evidence of healing, while 4 were accepted as "control" lesions.

Twenty-one of the 27 "closed" necrotic or healed lesions from 11 of 15 patients and all "control" lesions yielded growth of *Mycobacterium tuberculosis* by the cultural techniques used in this study. Thus, viable tubercle bacilli were recovered from approximately 78 per cent of the "closed" or healed lesions cultured during this investigation, or from approximately 73 per cent of the small series of patients from whom such lesions were obtained. D'Esopo, in contrast, observed growth of *M. tuberculosis* in cultures of only 35 per cent of patients from whom such lesions were obtained after preoperative treatment with twice-weekly streptomycin and para-aminosalicylic acid.

The impression has been gained that D'Esopo and his associates may frequently have used treatment periods in excess of eight and possibly in excess of twelve months. The statement has been made by them, however, that the patients whom they have selected for resection were mostly ones who had been treated "with an arbitrary minimum period of four months of preoperative chemotherapy." It has been pointed out, furthermore, by these investigators that the frequency with which viable tubercle bacilli may be demonstrated in lesions from patients who have had prolonged chemotherapy is lower in those who receive daily than in those who receive twice-weekly streptomycin (3). All patients described in the present report received more than four months of chemotherapy; a number received more than eight months, although none received more than twelve months of chemotherapy. None of the patients in the

present series received streptomycin on a daily basis during the preoperative period.

From the data presented it is apparent that, with improved cultural techniques, the frequency with which viable strains of *M. tuberculosis* may be recovered from "closed" or healed pulmonary lesions is significantly increased. The long preliminary incubation period in liquid medium required to demonstrate growth of the organisms from certain of the lesions emphasizes the low metabolic state in which they presumably might survive within the host. One would anticipate that, even if the number of bacilli were small, actively metabolizing microbial cells would multiply sufficiently within an incubation period of nine weeks in a presumably optimal medium to permit detection of such growth. No evidence is available, however, to prove if, in the present study, growth of tubercle bacilli ever occurred in the liquid medium used. Even after removal from their *in vivo* environment with its various suppressive influences, the organisms appear in many instances to continue in a suppressed state. Actual multiplication may well have resulted only after transfer of nonmultiplying surviving cells to an appropriate solid medium at the opportune time. There is little reason to suppose from the observations presented herein that the long incubation period necessary before growth became apparent was due only to an unusually slow generation time. One cannot help but wonder if the situation may not be analogous to a stage of dormancy.

The possibility that the viable cells detected in only a portion of the culture tubes inoculated with certain of the specimens, and then only after prolonged incubation, might be due to contamination with *M. tuberculosis* cannot be overlooked. The care taken to maintain sterile precautions throughout the study and to reduce to a minimum the possibility of contamination has been discussed in the foregoing report. Bacterial contamination with nonacid-fast bacteria occurred in only 2 of more than 500 culture tubes (or flasks) utilized in the present study during the past twelve months. Whereas mold contamination resulted somewhat more frequently, the incidence was less than 1 per cent. It seems unlikely that contamination with virulent tubercle bacilli could account for the high incidence with which growth of this species of microorganism has been observed in cultures of lesions from patients with pulmonary tuberculosis.

The data presented herein suggest that tubercle bacilli may remain viable in apparently "closed" necrotic or healed lesions and that the viability of these organisms can be demonstrated in many instances. The particular lesions studied were all derived from patients who had been receiving extensive chemotherapy on regimens widely employed today. Although the number of cells surviving within the fibrocaseous areas may indeed be few, in some instances at least, it is apparent that sterilization of the lesions apparently has not occurred. Furthermore, although data are available to prove that streptomycin and isoniazid may penetrate into caseous tuberculous lesions, no such data are available with respect to para-aminosalicylic acid. In addition, there is no evidence which indicates whether or not any of these agents, either singly or in combination, are capable of exerting antimicrobial effects under the conditions prevalent

within the lesion; indeed, it is likely that one or another of them may be totally ineffective as antimicrobial agents against microorganisms existing in such an environment.

The various factors which conceivably may act to prevent sterilization of lesions within the host have been discussed in a recent review by McDermott (29), who has offered three possible explanations for the phenomenon:

> . . . (*1*) that the microorganisms which survive represent the more drug-resistant fraction of the total infecting population; (*2*) that certain of the parasites are so situated within the body that they are not reached by the antimicrobial drug; and (*3*) that certain members of the microbial population are subsisting at such a low level of metabolic activity throughout the entire period of chemotherapy that they are physiologically insusceptible to the action of the drug.

Although the strains of *M. tuberculosis* isolated in the present study are highly susceptible to the *in vitro* antimicrobial activity of streptomycin and isoniazid, it is not inconceivable, as pointed out by McDermott, that such susceptibility may *not* represent the situation with respect to these organisms when in a metabolically suppressed state within the host.

In reference to what he designates physiologic insusceptibility, he states:

> Without question a most attractive hypothesis to explain post-treatment relapse is the notion that, during the entire period of therapy, certain of the parasites are subsisting at such a low level of metabolic activity that they fail to be affected by what are usually inhibitory quantities of a particular drug.
>
> Such a hypothesis implies three propositions:
>
> (*1*) At the time of initiation of therapy heterogeneity exists (or is thereby induced) in the degree of metabolic activity of individual parasites.
>
> (*2*) There is reason to believe that bacteria can subsist at a level of metabolic activity so low that they are 'indifferent' to the presence of the drug.
>
> (*3*) Bacteria which are capable of multiplying at intervals of minutes or hours can survive within the body without a significant degree of multiplication for periods measurable in weeks or months in a manner analogous to the behavior of spores (29).

The presence of viable microorganisms in any lesion within the host implies, of necessity, the possibility that ultimately these parasites may regain their ability to metabolize actively and that they then may bring about relapse. This situation is not unique to tuberculosis.

> If relapse represents, however, merely a failure to carry a drug-parasite reaction to completeness (i.e., a failure in delivery of drug), it presumably could be attacked and prevented by appropriate manipulations of the time-dose relationships of the drug therapy. On the other hand, if relapse occurs because a variety of types of drug-parasite relationships is coexisting simultaneously, only some of which lead to death of the parasite, its prevention would require other measures. These would presumably consist either of drastic prolongation of chemotherapy to a period measurable in months or years, or of attempts to develop methods for enhancing the resistance of the host to the infection in question (29).

In this connection, the possibility of the development of more effective chemotherapeutic agents should also be included. By time-dose alterations of present

chemotherapies, however, it has not been possible, except in infections caused by meningococci, gonococci, and vivax malaria, to abolish completely relapse or the "carrier" state in any susceptible infection (29).

It is conceivable that future studies may indicate that certain chemotherapeutic regimens may be more effective than others in sterilizing tuberculous pulmonary lesions in man. Evidence in favor of any single drug or combination of drugs, or in favor of any single chemotherapeutic regimen, however, is not yet available.

It is conceivable that future studies may develop chemotherapies of a qualitatively different sort than the suppressive type of drugs now available. The demonstration that chloraquine together with primaquine appears to be an eradicative type of therapy in vivax malaria in man and the recent observations on pyrazinamide–isoniazid in tuberculous mice would support this thesis (26).

It is highly significant that tubercle bacilli can be shown to survive in necrotic tuberculous pulmonary lesions, even after long-term chemotherapy, and can eventually emerge *in vitro* after months of incubation. Although in many instances the surviving microbial cells appear to be in a suppressed metabolic state, if such a state exists, it is obviously reversible. Evidence is lacking to indicate the extent to which chemotherapy may be responsible for this effect. It is not inconceivable that this may be a general phenomenon occurring in any semi-healed tuberculous lesion, and the implications with respect to relapse of tuberculosis are obvious.

SUMMARY

Microbiologic observations on 31 resected tuberculous lesions from 19 patients are reported herein. *M. tuberculosis* was recovered from 25 of the 31 lesions, or from 15 of the 19 patients. In the case of 9 of the 25 lesions from which viable tubercle bacilli were isolated, by the cultural methods described, viability was first detected only after prolonged incubation for more than nine to twelve weeks. All strains of *M. tuberculosis* recovered from these lesions were highly virulent for guinea pigs, although only 10 of the 25 lesions showing growth were capable of producing tuberculosis on direct inoculation into guinea pigs.

Fourteen of the 19 patients had received four to twelve months of chemotherapy preoperatively and showed morphologic evidence of cavity closure. One had received eleven months of chemotherapy preoperatively and showed evidence of an open cavity with healing. Strains of *M. tuberculosis* were grown from 21 of the 27 "closed" or healed lesions from these 15 patients, or from 11 of the 15 patients. Although prolonged incubation was necessary for detection of viable tubercle bacilli in lesions from patients who had had repeated courses of chemotherapy, e.g., from "re-treatment" cases, no correlation was observed between the length of the preoperative course of chemotherapy or the period of preoperative noninfectiousness and the frequency with which viable tubercle bacilli were detected.

Growth of *M. tuberculosis* was readily demonstrated on cultivation of 4

lesions from "control" patients who either had had no preoperative chemotherapy or had patent open cavities at the time of resection.

From the data presented, it is apparent that tubercle bacilli can survive in healed or semi-healed necrotic pulmonary lesions, even after prolonged chemotherapy, and that, in many instances, their viability can be demonstrated by appropriate cultural techniques.

SUMARIO

La Aparición Tardía de M. Tuberculosis en Cultivos Líquidos de Lesiones Pulmonares Resecadas de Seres Humanos

En el presente trabajo se informa de las observaciones microbiológicas efectuadas en 31 lesiones tuberculosas resecadas de 19 pacientes. En 25 de las 31 lesiones, o sea en 15 de los 19 pacientes, se halló *M. tuberculosis*. En el caso de 9 de las 25 lesiones de las cuales se aislaron bacilos tuberculosos viables mediante los métodos de cultivo descritos, sólo pudo determinarse primero la viabilidad de los gérmenes después de incubación prolongada durante más de 9 a 12 semanas. Todas las cepas de *M. tuberculosis* obtenidas de estas lesiones fueron sumamente virulentas para los cobayos, aunque sólo 10 de las 25 lesiones que presentaban desarrollo fueron capaces de producir tuberculosis por inoculación directa en cobayos.

Catorce de los 19 pacientes que fueron objeto del presente estudio habían sido tratados durante 4 a 12 meses mediante quimioterapia preoperatoria y presentaron pruebas morfológicas de cierre de cavidades. En uno que había sido sometido a la quimioterapia preoperatoria durante 11 meses se hallaron manifestaciones de cavidad abierta con cicatrización. Se obtuvieron cepas de *M. tuberculosis* de 21 de las 27 lesiones "cerradas" o cicatrizadas de estos 15 pacientes, o sea de 11 de los 15 enfermos. Aunque fue necesario hacer incubación prolongada para descubrir bacilos tuberculosos viables en las lesiones de pacientes que habían sido sometidos a repetidas series de quimioterapia (es decir, de casos de tratamiento reiterado), no se observó correlación entre la duración de la serie quimioterápica preoperatoria o el período no infeccioso de la fase preoperatoria y la frecuencia con que se descubrieron bacilos tuberculosos viables.

El desarrollo de M. tuberculosis se demostró fácilmente al hacer el cultivo de 4 lesiones de pacientes "testigos" que no habían recibido quimioterapia preoperatoria o bien tenían francas cavidades abiertas en el momento de la resección.

De los datos presentados se desprende manifiestamente que los bacilos tuberculosos pueden sobrevivir en lesiones pulmonares necróticas cicatrizadas o semicicatrizadas, incluso después de quimioterapia prolongada, y que, en muchos casos, puede demostrarse su viabilidad mediante técnicas de cultivo apropiadas.

RESUME

Apparition tardive de M. tuberculosis dans les cultures liquides de lesions pulmonaires réséquées chez l'homme

Cette communication se réfère aux observations microbiologiques faites sur 31 lésions tuberculeuses réséquées à partir de 19 malades. Le *M. tuberculosis* fut obtenu dans 25 sur les 31 lésions, à savior chez 15 des 19 malades. Dans le cas de 9 de ces 25 lésions à partir desquelles des bacilles de Koch viables furent isolés par les méthodes de culture décrites, la viabilité ne fut déterminée qu'après incubation prolongée pendant plus de 9 à 12 semaines. Toutes les souches de *M. tuberculosis* obtenues de ces lésions étaient extrêmement virulentes pour les cobayes, quoique seulement 10 des 25 lésions en cours de croissance étaient capables de produire la tuberculose après inoculation directe dans ces animaux.

Quatorze des 19 malades faisant l'objet de cette étude avaient été soumis à une chimiothérapie préopératoire pendant 4 à 12 mois et présentaient des signes morphologiques de

fermeture cavitaire. Un de ces malades, chez qui la chimiothérapie préopératoire avait été appliquée pendant 11 mois, présentait des signes d'une cavité ouverte en voie de cicatrisation. Des souches de *M. tuberculosis* furent obtenus à partir de 21 des 27 lésions "fermées" ou cicatrisées chez ces 15 malades, c'est-à-dire chez 11 d'entre eux. Bien qu'une incubation prolongée fût nécessaire pour déceler des bacilles de Koch viables dans les lésions des malades qui avaient subi des séries répétées de traitements chimiothérapiques (c.à.d. dans les cas ayant reçu un "retraitement"), aucune corrélation ne fut observée entre la durée du traitement chimiothérapique préopératoire ou la période non infectieuse de la phase préopératoire et la fréquence avec laquelle il fut possible de découvrir des bacilles de Koch viables.

On a facilement pu démontrer la *croissance de M. tuberculosis* en préparant des cultures à partir de 4 lésions dans des malades servant de "témoins," chez lesquels aucune chimiothérapie préopératoire n'avait été pratiquée ou qui avaient des lésions manifestement ouvertes lors de la résection.

De ces données il est évident que les bacilles de la tuberculose peuvent survivre dans les lésions pulmonaires nécrotiques tant cicatrisées que semicicatrisées, même après une longue période de chimiothérapie, et que, dans beaucoup de cas, leur viabilité peut être démontrée par des techniques de culture appropriées.

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INDEFINITELY PROLONGED CHEMOTHERAPY FOR TUBERCULOSIS[1]

Preliminary Report

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INTRODUCTION

The value of antimicrobial therapy in tuberculosis is unquestioned. It is also generally held that an adequate regimen should include more than one agent (1). Streptomycin, para-aminosalicylic acid (PAS), and, more recently, isonicotinic acid compounds have been given according to various schedules. Initially, streptomycin alone was administered intensively for periods of six weeks to four months (2, 3). Para-aminosalicylic acid alone was given for varied periods of time (4). Thereafter, improved therapeutic effects and laboratory results indicating delayed emergence of drug-resistant organisms led to the introduction of combined therapy (5). With isonicotinic acid compounds the advantages and disadvantages of therapy with a single drug were again demonstrated (6). It has now become evident that combinations of streptomycin, PAS, and/or isoniazid should be used in most instances. It is also becoming clear that clinical results improve as therapy is prolonged. However, the duration of treatment, the tolerance of the patient for prolonged therapy, and the long-term toxicity of the drugs have not been established. In an attempt to evaluate these factors, the following group of 52 patients who received prolonged combined antimicrobial therapy is reported.

MATERIAL

The patients were unselected. They represented all patients with pulmonary tuberculosis who were being treated with drugs for the first time and who had received combinations of antimicrobial agents for more than one year at the time of review. The ages of the patients ranged from fourteen to sixty-four years. There were 6 minimal, 37 moderately advanced, and 6 far advanced cases of active pulmonary tuberculosis and 3 cases of active predominantly extrapulmonary tuberculosis. Treatment was continuous for thirteen to thirty-two months and in most instances is still being maintained. As indicated in the tables, the therapeutic regimens varied depending on clinical indications, and included combinations of the three agents: streptomycin sulfate, sodium para-aminosalicylic acid (PAS), and isoniazid. With the exception of the patient with tuberculous meningitis, to whom daily injections were administered for thirteen months, daily streptomycin was not continued longer than five months; usually streptomycin was given daily for one to three months, after which interrupted therapy two or three times weekly was continued through the period of observa-

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tion. The dosage of isoniazid was usually 4 mg. per kg. of body weight per day. Sodium PAS was given in dosages of 12 to 15 gm. daily.

RESULTS

It will be seen (table 1) that, in the patients with minimal tuberculosis with tubercle bacilli in the sputum at the time treatment was begun, sputum "conversion" was attained within one month. The clinical response and roentgenographic improvement were excellent in all patients, there have been no relapses, and treatment has now continued for periods of fourteen to thirty-two months. Drug toxicity will be discussed later.

In the 37 cases of moderately advanced disease (table 2), 25 patients had cavity demonstrable by roentgenography, 13 had known endobronchial tuberculosis, and 36 had had sputum or gastric contents positive for tubercle bacilli; in 5 of the latter, the immediate pretreatment specimen was negative. The antimicrobial drugs were given for thirteen to thirty-two months, with a mean of twenty and one-tenth months at the time of review. In 28 of the 31 patients, sputum positive for tubercle bacilli on culture at the time therapy was instituted became negative in periods ranging from less than one month to twelve months. In 3 patients the sputum became negative for tubercle bacilli only after pulmonary resection. None of the entire group has had clinical or roentgenographic relapse, but 7 have had one or more cultures positive for *M. tuberculosis* eight to twenty months after the commencement of treatment. The sputum of 3 of these patients again became positive after drug treatment had been stopped. Excisions of lobes or segments were carried out in 8 patients.

Of the 6 patients with far advanced disease (table 3), 5 with definite cavitation and all with sputum positive for tubercle bacilli, all showed good therapeutic response and the sputum became negative for tubercle bacilli within four months. In 4, negative cultures persist to date (on continued chemotherapy); in 2, cultures positive for tubercle bacilli recurred fourteen and ten months after institution of therapy. None has shown clinical or roentgenographic evidence of relapse. The duration of continuous treatment in this group has been nineteen to twenty-nine months.

One case each of tuberculous meningitis, endobronchial tuberculosis without active parenchymal disease, and tuberculous pleuritis and pericarditis are included in table 1. These patients have been receiving drug therapy for eighteen to twenty-six months with good response in all and no evidence of relapse.

Drug Toxicity

Drug toxicity has been relatively slight. Temporary interruption of treatment, usually for a day or two, was carried out when PAS caused gastric discomfort or diarrhea, but substitution of another drug for PAS was necessary in only 3 instances. In one case, streptomycin was stopped because it appeared to aggravate a pre-existent psoriasis. All other patients were able to continue treatment.

The gastrointestinal side-effects of PAS, usually mild, occurred at some time or other in many patients who received this drug. With persistence of treatment,

TABLE 1

PROLONGED DRUG THERAPY OF EXTRAPULMONARY AND MINIMAL PULMONARY TUBERCULOSIS

CASE NUMBER	PRETREATMENT STATUS				DRUG TREATMENT			RESPONSE TO TREATMENT			RELAPSE	
	Stage	Cavity	Endobronchial Tuberculosis	Sputum/Gastric Washings	Drugs	Duration		Clinical and Roentgenographic	Sputum Conversion	Time of Conversion	Clinical or Roentgenographic	Sputum
						Each	Total					
						months				*months*		
22	Minimal	0	–	+	SP-I	3						
					SRP-I	11	14	Good	+	1	0	0
32	Minimal	0	–	+	SP-D	2						
					SP-I	21	23	Good	+	1	0	0
39	Minimal	0	–	0	SP-I		32	Good			0	0
45	Minimal	0	–	+0	SP-I		14	Good	+	Prior to therapy	0	0
51	Minimal	0	–	0	SP-I		18	Good			0	0
52	Minimal	0	–	0	SP-I		18	Good			0	0
31	Pericarditis and pleuritis	0	–	0	SP-D	3						
					SP-I	15	18	Good			0	0
16	Meningeal	0	–	0	SP-D	13						
					SP-I	13	26	Good			0	0
38	Minimal and endobronchial	0	+	+	SP-D	1.5						
					SP-I	19.5	21	Good	+	1	0	0

SP-I: Streptomycin or dihydrostreptomycin sulfate, interrupted, two or three times weekly, and PAS sodium.
SRP-I: Streptomycin or dihydrostreptomycin sulfate, interrupted, two or three times weekly, isoniazid, and PAS sodium.
SP-D: Streptomycin or dihydrostreptomycin sulfate and PAS sodium—daily.

TABLE 2

PROLONGED DRUG THERAPY OF MODERATELY ADVANCED PULMONARY TUBERCULOSIS

Total Number of Cases	37
Number with cavity	25
Number bronchoscoped	27
Number with endobronchial disease	13
Number with sputum or gastric washings positive for *M. tuberculosis* at initiation of therapy	31
Number with sputum previously positive for tubercle bacilli but negative at onset of therapy	5
Medication	
Number who received streptomycin and PAS alone*	27
Number changed to streptomycin and isoniazid	3
Number in whom isoniazid was added	5
Number who received streptomycin, PAS, and isoniazid from initiation of therapy	2
Results	
Number of sputum "conversions" on chemotherapy	28
Number of sputum "conversions" only after resection	3
Time of "conversion" to negative on chemotherapy	1–12 months
Number of bacteriologic relapses	7

* Two patients received PAS alone for three and four months respectively prior to combined therapy.

temporary discontinuance of medication, or temporary reduction of dose, the symptoms usually disappeared.

Patients who received streptomycin sulfate for long periods sometimes offered rather vague complaints on the days they received the drug (twice weekly in most instances). These included headache, malaise, a drunk or "hung-over" feeling, drowsiness, and circumoral paresthesias. Except for the paresthesias, these symptoms have not been emphasized by other observers. The present writers are observing this syndrome more and more often as treatment is prolonged. Patients refer to it as a "strep-y" feeling, and usually experience it only on days when they receive the drug.

No changes have been observed in renal or hepatic function or in the peripheral blood counts. Routine audiometric examinations have not been performed, but no gross diminution in hearing has been noted (except in the patient with tuberculous meningitis, to whom streptomycin was given daily for thirteen months and in whom damage to the eighth nerve by the inflammatory process itself cannot be excluded). Vertigo has not been troublesome, although these patients often showed loss of response to caloric stimulation when tested. With respect to isoniazid, toxicity has been infrequent. It should be emphasized that the dose of isoniazid rarely exceeded 4 mg. per kg. of body weight per day and that the period of observation with this drug has been shorter than with streptomycin and PAS. During therapy with isoniazid, acneform eruptions have been observed in a few patients; they subsided spontaneously. One psychotic episode has occurred in a patient apparently predisposed; it has not yet been established

TABLE 3

PROLONGED DRUG THERAPY OF FAR ADVANCED PULMONARY TUBERCULOSIS

CASE NUMBER	PRETREATMENT STATUS				DRUG TREATMENT			RESPONSE TO TREATMENT			RELAPSE		
	Stage	Cavity	Endobronchial Tuberculosis	Sputum/ Gastric Washings	Drugs	Duration		Clinical and Roentgenographic	Sputum Conversion	Time of Conversion	Clinical or Roentgenographic	Sputum	Time
						Each	Total						
						months				*months*			*months*
15	Far advanced	+	+	+	SP-I	4							
					SR-I	18	22	Good	+	4	0	0	
40	Far advanced	+	0	+	SP-I	1							
					SR-I	18	19	Good	+	1	0	+	10
41	Far advanced	+	−	+	SP-D	2.5							
					SP-I	1							
					S	1.5							
					SR	17	22	Good	+	3	0	+	14
42	Far advanced	±	−	+	SP-D	5							
					SP-I	21	26	Good	+	<1	0	0	
46	Far advanced	+	−	+	SP-I		22	Good	+	1	0	0	
53	Far advanced	+	0	+	SP-I		29	Good	+	*	0	0	

SP-I: Streptomycin or dihydrostreptomycin, interrupted, and PAS sodium.
SR-I: Streptomycin or dihydrostreptomycin, interrupted, and isoniazid.
S: Streptomycin or dihydrostreptomycin.
SP-D: Streptomycin or dihydrostreptomycin sulfate and PAS sodium—daily.
* Therapy instituted prior to admission.

whether it was related to the drug or was coincidental. Central nervous system and psychic effects are much less marked with isoniazid than with iproniazid, as previously reported from this hospital (7).

DISCUSSION

Relapse is distressingly frequent in all forms of tuberculosis. Modified bed rest alone, as applied at Trudeau Sanatorium during the years 1927 through 1946, failed in more than 27 per cent of patients with uncomplicated minimal pulmonary tuberculosis to prevent progression of the disease to an advanced state (8). An even worse record is made with patients having advanced disease at the time of diagnosis. Approximately one-third of the patients under treatment at Montefiore Hospital, New York, have at some time previously had treatment for their tuberculosis in a hospital; many have had collapse therapy, courses of chemotherapy, and even resection, yet "reactivation" was not prevented in these patients.

It is often difficult to predict which patients will remain well permanently, even after clinical, roentgenographic, and bacteriologic criteria of "stability" have been fulfilled and so-called "arrest" attained. Mitchell (9), in analyzing results of treatment of the solitary dense pulmonary focus ("tuberculoma"), reports a cumulative rate of progression of disease in 26.1 per cent when projected over twenty-two years by the life-table method. Although many patients are well enough to work in the intervals between exacerbations, long follow-up discloses costly interruptions of normal living among patients treated by all known methods. Relapses are now being observed in patients treated with antimicrobial agents for relatively short periods of weeks to several months during the years 1947–1951. Moreover, the writers are harvesting a disheartening crop of late relapses in patients treated with resection followed by only short-term chemotherapy.

In the light of these observations and accumulated experience with this disease, it seems to the writers that the time has come to explore very prolonged, perhaps life-long, treatment with antimicrobial agents in some patients. It is never certain that all tubercle bacilli have been removed from the tuberculous host when a grossly involved lobe or segment is excised, since foci not visible roentgenographically or palpable at the operating table are often present in the unresected segments, tracheobronchial tree, lymph nodes, or extrathoracic organs. Nor can complete sterilization of these sites be assumed following any arbitrary period of drug treatment. As a consequence, the writers have embarked on an experimental program of treatment, the duration of which will be limited for the time being only by patient acceptance, drug toxicity, and bacterial resistance. Excisional surgery and other procedures are performed as indicated, but drug administration is continued indefinitely through the period of in-hospital rehabilitation and beyond. The patients return to their homes, are permitted to resume work or schooling, and activity is gradually increased to full tolerance as in the past, but drug therapy is continued. When the regimen includes streptomycin, the patient can usually be taught the technique of intra-

muscular injection and performs it himself; when this is not feasible, visiting nurse services are utilized.

The preliminary observations reported herein on the first group so treated are gratifying and encourage further trial for longer periods in more patients. None of the entire group of 52 patients has yet suffered clinical or roentgenographic relapse while receiving the drugs. Patients with similar lesions who have received the same drugs for shorter periods of time frequently developed local extensions of their disease process and/or bronchogenic spread after drugs were stopped—sometimes promptly, more often after some months. It should be noted that this is true, also, of patients who receive chemotherapy for the *second* time, exacerbation sometimes occurring *during* treatment under these conditions. It is important, therefore, to re-emphasize the desirability of not instituting drug therapy unless the indications are clear-cut, for once started it is believed that it should not be discontinued without good reason and that treatment should be very prolonged, perhaps indefinitely prolonged.

The possible limiting factors to the regimen proposed are drug toxicity, patient acceptance, and bacterial resistance. The latter has not presented a problem to date, although both clinical and laboratory data over prolonged periods are needed. Drug side-effects are seen in greater numbers as treatment is prolonged, but to date, and up to three years of treatment, they have not been serious in comparison with the risks of the infection itself. The importance of close observation should be emphasized and chemotherapy should not be recommended for *any* period unless competent personnel and adequate facilities are available. Patient acceptance of all of the drugs for long periods has been satisfactory in the present experience. The patient's willingness to continue treatment for years is in proportion to the physician's conviction that it is necessary and to his transmission of his belief to the patient.

The occurrence of bacteriologic relapse, i.e., reappearance of tubercle bacilli in sputum or gastric contents after their prolonged absence, was seen under drug treatment in 6 of the 52 patients reported. The *degree* of positivity was reduced—indeed, only occasional cultures yielded bacilli. Nevertheless, this may be the forerunner of clinical relapse and indicates that the millenium of drug-sterilization has not been attained for the tubercle bacillus *in vivo*. Even in these patients, however, and in the group as a whole, the preliminary results are far better than with any previously available therapy for tuberculosis.

SUMMARY

Fifty-two patients with tuberculosis who had never before been treated with antituberculous drugs have received combinations of two or more of the drugs streptomycin, para-aminosalicylic acid (PAS), and isoniazid for periods ranging from thirteen to thirty-two months. Other therapies were carried out as indicated.

The clinical and roentgenographic results have been excellent, and no relapses have occurred in any of the 52 patients to the date of this report.

Tubercle bacilli reappeared in sputum or gastric contents of 6 patients while drug therapy was being continued.

In general, drug toxicity was slight and patient tolerance was good, making long-term therapy practicable. Attention is directed to a new syndrome of streptomycin intoxication.

The guarded long-term prognosis for patients with tuberculosis is discussed. In view of the difficulty of predicting which patients will remain well, exploration of long-term drug treatment of indefinite duration is proposed.

Establishment of optimal duration of therapy will require observation of large numbers of patients over periods of years. Further study is needed with special reference to patient acceptance, drug effects, relapse rates, and bacterial resistance.

SUMARIO

Quimioterapia Prolongada Indefinidamente para la Tuberculosis

Cincuenta y dos tuberculosos que jamás habían sido tratados antes con drogas antituberculosas han recibido combinaciones de de dos o más de éstas: estreptomicina, ácido para-aminosalicílico (PAS) e isoniacida durante períodos de tiempo que variaron de trece a treinta y dos meses. Otras terapéuticas fueron aplicadas conforme a las indicaciones para ellas.

Los resultados clínicos y radiográficos han sido excelentes, y no ha habido recidivas en ninguno de los 52 enfermos hasta la fecha de esta comunicación.

Mientras continuaba la farmacoterapia, reaparecieron bacilos tuberculosos en el esputo o el contenido gástrico de 6 enfermos.

En general, la toxicidad medicamentosa fué leve y la tolerancia de los pacientes buena, permitiendo esto administrar tratamiento a largo plazo. Llámase la atención sobre un nuevo síndrome de intoxicación estreptomicínica.

Discútese el pronóstico reservado a largo plazo para los tuberculosos. Visto lo difícil que es predecir qué enfermos permanecerán bien, se propone una exploración a largo plazo de la farmacoterapia de duración indefinida.

El establecimiento de la duración óptima del tratamiento requerirá la observación de grandes números de enfermos durante años enteros. Se necesitan estudios ulteriores con referencia particular a la aceptación de enfermos, los efectos de las drogas, las tasas de recidivas y la resistencia bacteriana.

RESUME

La chimiothérapie indéfiniment prolongée dans la tuberculose

Cinquante-deux malades atteints de tuberculose et qui n'avaient jamais été traités par les agents antituberculeux ont reçu un traitement combiné comprenant deux ou plusieurs de ces médicaments: streptomycine, acide para-aminosalicylique (PAS) et isoniazide pendant des périodes variant entre treize et trente-deux mois. D'autres traitements ont été appliqués selon les indications.

Les résultats cliniques et radiologiques ont été excellents, et, à ce jour, aucun des malades sur cinquante-deux sujets étudiés n'a présenté de rechute.

Les bacilles tuberculeux ont fait leur réapparition dans les crachats ou les liquides de lavage gastrique chez six malades en cours de traitement.

En général, la médication s'est montrée peu toxique et a été bien tolérée par les malades, ce qui a permis le traitement de longue durée. Les auteurs attirent l'attention sur un nouveau syndrome d'intoxication par la streptomycine.

Le pronostic éloigné réservé en tuberculose est commenté. En raison de la difficulté de prédire quelsera celui des malades dont la guérison se maintiendra une exploration des cures à long terme, de durée indéfinie est suggérée.

La détermination de la durée optima de la thérapie réclame l'observation d'un grand nombre de malades pendant des années. Il est nécessaire de poursuivre les recherches, surtout en ce qui concerne l'acceptation de ce traitement par les malades, l'action des drogues, le taux de rechute et la résistance bactérienne.

Addendum

The 52 patients who are the subject of this report have now been followed *on chemotherapy* for periods of thirteen to thirty-eight months: 22 of them for thirteen to twenty-four months, 28 for twenty-five to thirty-six months, one for thirty-seven months, and one for thirty-eight months. The mean duration of chemotherapy in the series to this date is twenty-five and four-tenths months. Significant drug toxicity other than that discussed above has not developed, and it has not been necessary to discontinue treatment because of side effects. Relapse or spread of disease has not yet occurred in any of the group.

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THE COURSE OF PULMONARY TUBERCULOSIS DURING LONG-TERM SINGLE-DRUG (ISONIAZID) THERAPY[1,2]

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INTRODUCTION

In the early studies of isoniazid, it was shown that the drug exerted impressive antituberculous activity in humans with pulmonary, miliary, or meningeal tuberculosis (1, 2, 3, 4, 5, 6, 7). It was also shown that isoniazid-resistant tubercle bacilli could be isolated with ease from tuberculous patients whether or not they had ever been treated with the drug (8). Following these initial demonstrations, two principal questions remained unanswered: the degree of antimicrobial activity of isoniazid compared with that of other antituberculous drugs; and the total length of the period during which isoniazid would remain effective in an individual patient.

Information on the first of these questions was provided by three types of study. In miliary-meningeal forms of tuberculosis, it was shown by the present writers that relapse did not occur during isoniazid therapy nor did meningitis make its first appearance once isoniazid therapy was started (7). Although the last-named phenomenon might be merely a reflection of the extent of distribution of the isoniazid (3), it could equally as well represent a greater degree of activity of the drug. Forty-week studies conducted under the auspices of the United States Public Health Service in a large group of patients with pulmonary tuberculosis showed that isoniazid was equal to streptomycin-PAS in therapeutic effectiveness (9). Finally, studies in the present laboratory by means of a precise quantitative technique for enumeration of *M. tuberculosis* within tissues have shown that populations of tubercle bacilli within the lungs or the spleens of mice are suppressed to a much greater extent by the administration of isoniazid than by either streptomycin alone or streptomycin-PAS adminis-

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tered together. These studies which are to be reported elsewhere in detail (10) have already been published in summary form (11, 12).

It seemed clear, therefore, that, at least during the early stages of drug administration, the antituberculous activity of isoniazid was equal to that exerted by streptomycin-PAS. Moreover, in the two situations which permitted the most satisfactory quantitative comparisons, namely miliary tuberculosis and the mouse population studies, isoniazid was clearly superior to streptomycin or to streptomycin-PAS.

After these initial demonstrations the second question, i.e., the duration of isoniazid effectiveness in an individual patient, remained to be considered. On this question there was by no means universal agreement among the various groups of investigators who were evaluating isoniazid. Some investigators (13, 14, 16, 17–24, 26, 27, 29, 30, 30*b*) were impressed by the ease with which isoniazid-resistant strains of tubercle bacilli could be isolated from patients during therapy. They either reported (14, 17, 19, 20, 22, 30*b*) or predicted (13, 15, 16, 26, 27) a correlation between such discharge of drug-resistant bacilli and an unfavorable course of pulmonary tuberculosis during isoniazid therapy. It was concluded, therefore, that the total duration of the period of general effectiveness of isoniazid when used alone was short; in some series (14, 19, 20, 22, 30*b*) possibly as short as two or three months in an appreciable percentage of treated patients. As a consequence, it was strongly recommended that isoniazid should never be administered alone, but always in conjunction with streptomycin or some other antituberculous drug (14, 16–22, 24–30). The prevalence of this opinion became so widespread that very few studies have been conducted on the long-term use of isoniazid alone in patients with pulmonary tuberculosis.

Belief that isoniazid usually had only short-term usefulness was not shared, however, by all groups of investigators. At least two groups, the USPHS investigators and the present writers, were sufficiently impressed with the accumulating evidence to the contrary to continue studies with isoniazid alone while conceding that multiple therapy might eventually be proved to be of even greater effectiveness (7, 9*a*). Three sorts of observations were responsible for this conclusion.

First was the fact that no instance of relapse of miliary tuberculosis was observed during prolonged isoniazid therapy (7). This was in distinct contrast to the previous experience with streptomycin (31). Second, it was reported in May, 1952 (4) that the problem of the significance of isoniazid-resistant tubercle bacilli was much more complicated than had been the case with streptomycin. The principal basis for this belief were the facts: that isoniazid-resistant tubercle bacilli, unlike their streptomycin counterparts, exhibited biologic changes in addition to those directly related to drug susceptibility; and that isoniazid-resistant tubercle bacilli could be isolated with ease from any patient discharging bacilli, whether or not he had received isoniazid. It seemed clear, therefore, that it would be necessary to develop more precisely quantitative cultural techniques than those customarily employed in order to establish the occurrence of population shifts from the drug-susceptible to the drug-resistant state which were meaningful in terms of loss of drug effectiveness. Finally, in the

forty-week USPHS studies in pulmonary tuberculosis, the incidence of disease progression during therapy was not significantly different among three groups of patients who received one or another of the three regimens of isoniazid alone, isoniazid-streptomycin, or streptomycin-PAS (9*a*, 9*b*). If isoniazid had only short-term effectiveness, its use as sole chemotherapy for a nine-month period should have yielded appreciably more relapses than occurred with streptomycin-PAS unless the short-term action were truly unprecedented in degree.

As a consequence of this interpretation of the evidence concerning isoniazid, the present investigators continued to use isoniazid as the sole chemotherapy in a consecutively observed series of patients with pulmonary tuberculosis whose treatment was started during the period from November, 1951, through August, 1952. Because of the paucity of observations on the long-term use of isoniazid alone, the course and outcome of the pulmonary tuberculosis in this group of patients form the basis of the present report.

MATERIALS AND METHODS

Type of tuberculous lesions: Roentgenographic abnormalities with the characteristics of tuberculous lesions were present in the chest films of all patients. In all but 3 of the 47 patients the pulmonary lesions at the start of therapy were designated *far advanced* or *moderately advanced* by the 1950 classification of the National Tuberculosis Association.

Previous chemotherapy: Twenty-five of the 47 patients had never received streptomycin and an additional 13 had received none within three months of the start of isoniazid. The other 9 patients had received various amounts of streptomycin, but for the most part had been treated for only four to six weeks when it was discontinued at the time isoniazid was started. Three patients in the total series received, during the first four weeks of isoniazid therapy, a thiosemicarbazone (32) subsequently shown to have little, if any, activity.

Measurement of roentgenographic changes: Serial roentgenograms were interpreted periodically, and changes were recorded using the customary categories of *marked improvement, moderate improvement, slight improvement, no change,* and *worse.*

For the purposes of the present report these categories have been reduced to three: *substantial improvement* (marked or moderate); *no significant improvement* (slight improvement or no change); and *worse.*

Microbiologic studies: M. tuberculosis was cultured from sputum or gastric washings of all but 4 of the 47 patients. In the 4 patients with sterile cultures, microscopic examination of stained smears of sputum revealed acid-fast bacilli at the time antimicrobial therapy was started or when cultures positive for *M. tuberculosis* had been obtained at some time prior to the use of isoniazid.

After the start of treatment, specimens for culture were obtained from all patients at approximately four-week intervals. In addition, during two separated weeks in June, 1953, and the first week in November, 1953, sputum and gastric washings from all patients were stained for acid-fast bacilli and cultured for *M. tuberculosis.*

Isoniazid-susceptibility in vitro: Determinations of isoniazid-susceptibility *in vitro* were conducted in liquid medium (Tween®-albumin) after primary isolation on solid medium (oleic-albumin-penicillin or Petragnani). The techniques used are described in detail in another report from this laboratory (33).

General management: All patients were hospital inpatients who were kept on bed rest with some individual modifications such as the allowance of bathroom privileges.

Isoniazid regimen: Isoniazid[5] was administered in a total daily dosage of 5 mg. per kg. of body weight given orally in two equally divided doses.

[5] Generously supplied as Nydrazid by E. R. Squibb & Sons Division; Mathieson Chemical Company, New York.

OBSERVATIONS

Forty-seven patients with pulmonary tuberculosis, moderately advanced or far advanced in extent, were started on isoniazid therapy with the expectation that it would be continued for at least one year. *Any patient whose therapy was altered in any way during the year, either by a change in drugs or by the use of collapse or excisional therapy, was eliminated from the study at that time.* The isoniazid therapy was continued unaltered only when, in the opinion of the observers, no other therapy could be expected to improve the patient's situation. Hence the results are to be regarded in terms of what happened to a group of patients started on a planned year of isoniazid who were subjected to continuous scrutiny with respect to changes in that therapy.

TABLE 1

LONG-TERM SINGLE DRUG (ISONIAZID) THERAPY

	Pulmonary tuberculosis
Number of patients started	47
Eliminated during year	15
Completed 1 year	32
Reasons for elimination before end of year	
Chemotherapy changed	
Drug resistance	2
Lack of improvement	2
Collapse or excisional procedures	6
Transferred elsewhere	3
Discharged against advice	2
	15

Success in Completing One Year of Therapy (Table 1)

Forty-seven patients were started on the year's regimen of isoniazid alone and 32[6] completed it with no alteration in therapy. An additional 3 patients who had experienced substantial roentgenographic improvement on isoniazid were changed to other chemotherapy on transfer to other hospitals. This occurred at three months in one case and at nine months in 2 cases.

The other 12 eliminations from the single-drug regimen occurred principally in the two middle quarters of the therapeutic year and were made for the following reasons:

In the first quarter, all patients continued on the single-drug regimen.

In the second quarter, 5 patients were eliminated: 2 by deliberate change in chemotherapy, 2 by surgery, and one by self-discharge.

In the third quarter, 6 more patients were eliminated from the series: one by

[6] Includes 3 patients who received between eleven and twelve months of therapy.

change in chemotherapy, 4 by collapse or excisional therapy, and one by self-discharge.

In the fourth quarter, only one patient was eliminated, and the cause was a deliberate change in chemotherapy.

Thus, approximately two-thirds of the group of 47 patients who started on the planned year of isoniazid therapy completed the year without alteration of therapy.

Clinical Course

The customary clinical observations were made of the patients' temperatures, weight changes, and statements concerning subjective status. These observations will not be reported, however, for they are in no way different from the corresponding observations in other forms of tuberculosis treated with isoniazid (1, 7) or with other antimicrobial drugs.

Course of Roentgenographic Abnormalities

The course of roentgenographic abnormalities throughout the year may be seen in table 2.

Only 6 of the 47 patients developed roentgenographic changes which could be classified as worse at any time during the one-year period (plates I–VI). Two of the 6 were eliminated from the series in the third and fourth quarters as mentioned above. Both received other chemotherapy.

Substantial roentgenographic improvement occurred in 29 of the 47 patients at some time or other throughout the year. At the year's end, 21 of the 32 patients had lesions designated on roentgenographic classification as having shown

TABLE 2

ROENTGENOGRAPHIC COURSE OF 47 PATIENTS ON ISONIAZID ALONE FOR ONE YEAR PERIOD

Expressed as number of patients with stated designation at various time periods

	0 MONTHS	3 MONTHS	6 MONTHS	9 MONTHS	12 MONTHS
Substantial improvement		21	24	23	21
No significant improvement		25	16	11	7
Worse		1	3	5	4
Cumulative number removed from series		0	4	8	15
Total		47	47	47	47

Summary of changes

Substantial (marked or moderate) improvement		29
No significant (slight or no change) improvement		12
Worse		6
Patients who completed year		32
Substantial improvement	21	
Cavity closure (all cavities)	12	

substantial improvement. There was only one instance of change of a patient's lesion from *substantial improvement* to *worse* and this consisted of the slight enlargement in size at six months of a cavity present at the start of treatment.

Cavity closure: Cavity closure (all cavities of each patient) occurred in 15 of the 44 patients with cavitary disease. In 7 of the 15 the closures did not occur until the third or fourth quarters of the year of therapy. As would be anticipated, cavity closure occurred principally in the patients who had received no previous antimicrobial therapy. Eleven of the 15 patients whose cavities closed had never received streptomycin.

Worsening during isoniazid therapy: In 6 of the 47 patients, roentgenographic change designated as "worsening" occurred at some time following the start of isoniazid therapy. In 5 of the 6 cases the change for the worse consisted of either enlargement of cavities previously present, first appearance of cavity in a previously abnormal density, or enlargement of a previously present area of infiltration. In the sixth case, a previously uninvolved area of lung was the site of a transient pneumonic process in association with some evidence of a nontuberculous bacterial infection of the lung. With this exception, in the entire series of 47 patients, there was no instance of the development of a totally new area of disease in a region of the lung previously uninvolved and definitely separated from the original lesions.

In several of the 6 cases which were classified as roentgenographically *worse*, it is not certain that the tuberculous lesions in question were actually "worse." In one, an annular shadow appeared on roentgenography and tomography in an otherwise regressing lesion but a nonexcavated healing lesion was found on resection (plate I). In another, a small solid lesion behind the clavicle showed a slight but definite increase in size during the first four months of isoniazid therapy and then stabilized for the remainder of the year of treatment (plate III). Three months after treatment had been stopped, the density started to increase in size and continued to enlarge during a short period of streptomycin-PAS therapy prior to resection. A third case was the one mentioned in the previous paragraph (plate II). The patient was an asthmatic with chronic upper lobe tuberculosis who developed an area of pneumonia in the right lower lobe in a setting suggestive of a pneumococcal pneumonia. The right lower lobe density did not completely disappear until sometime between twenty-one and forty-nine days after its appearance. Consequently, it is not possible to be certain whether it represented a slowly resolving pneumococcal pneumonia in an asthmatic or a rapidly resolving tuberculous process. The details and illustrative roentgenograms of all 6 of these cases designated as roentgenographically worse are presented in plates I to VI, inclusive.

Influence of previous chemotherapy on the isoniazid-treated patients: Approximately one-half of the group (23 of 47 patients) had received streptomycin at some time prior to the expected year's treatment with isoniazid. Less than one-half of this number, however, i.e., 9 patients, had received any streptomycin therapy within the three months immediately preceding the start of the isoniazid. Thus, three-quarters of the group either never received streptomycin

or had received none for more than three months prior to the start of the isoniazid therapy. It is not surprising, therefore, that, except for cavity closure, there were no conspicuous differences with respect to roentgenographic changes between previously treated and previously untreated patients.

Change in chemotherapy within one year of the start of isoniazid therapy: In 4 of the 47 patients, the chemotherapy regimen of isoniazid alone was altered in an attempt to provide more satisfactory therapy. In 2 of these patients the drug changes were made in the fourth month because of unchanged roentgenographic findings and the isolation of drug-resistant tubercle bacilli (6 to 12 and 25 to 50 γ of isoniazid per ml.). In the other 2 patients the therapy was changed at seven months and at nine months, at which times the roentgenographic course was classified as "worse" (plates I and II).

The first 2 of these cases in which treatment was altered after only three to four months may well represent instances of too hasty change of therapy. Both patients were treated early in the course of the study. The other two cases represented instances of cavitary disease which had persisted despite seven to nine months of isoniazid therapy. One of the patients (plate VI) had experienced considerable roentgenographic improvement despite the persistence and reappearance of cavities; the other patient is the asthmatic with the transient pneumonia mentioned above (plate II). In one instance, the patient's strain of tubercle bacilli required isoniazid concentrations of approximately 2 or 3 γ per ml. for inhibition; the other patient's strain was completely drug-susceptible.

The subsequent course of the disease in these 3 patients following the institution of other antimicrobial therapy was not substantially different from what had been observed during the period of isoniazid therapy. Successful resectional procedures were eventually performed in 3 of the 4 patients.

Summary of roentgenographic changes: In crude terms, therefore, substantial roentgenographic improvement occurred in two-thirds of the 47 patients at some time during the year, and roentgenographic worsening occurred in less than one-fifth. Cavity closure (all cavities per patient) occurred in approximately one-third of those with cavitary lesions. When significant roentgenographic improvement did occur, it was maintained either throughout the entire year or until definitive surgery, with the exception of the one slight increase in cavity size mentioned above. Of the 32 patients who completed the year's treatment, substantial roentgenographic improvement had occurred in 21.

Presence of M. Tuberculosis in Sputum or Gastric Washings During One Year of Therapy

At the start of therapy, tubercle bacilli could be cultured from sputum or gastric washings of 43 of the 47 patients. After one year of isoniazid therapy, tubercle bacilli could still be cultured from 11 patients. This number represented approximately one-quarter of the original group who were demonstrably infectious at the start of treatment or one-third of all patients who completed the year of therapy.

The cultural studies in terms of months after the start of therapy are as follows:

At three months, tubercle bacilli were cultured from 24 of 47 patients, or 50 per cent of the group.

At six months, tubercle bacilli were cultured from 17 of 41 patients, or 41 per cent of the group.

At nine months, tubercle bacilli were cultured from 13 of 35 patients, or 38 per cent of the group.

At twelve months, tubercle bacilli were cultured from 11 of 32 patients, or 33 per cent of the group.

There were 4 patients from whom tubercle bacilli were not cultured immediately before the start of isoniazid therapy. When these 4 are excluded, the actually demonstrated incidence of reversal of infectiousness is 45 per cent at three months and 58 to 66 per cent at six, nine, and twelve months. It must be remembered, however, that 12 patients were eliminated from the series during the year because of a change in drug or other therapy. If it be assumed that these 12 patients would have continued to discharge tubercle bacilli had they continued to receive only isoniazid, the incidence of reversal of infectiousness would have been approximately 50 per cent.

In general, once *M. tuberculosis* disappeared from sputum and gastric washings of an individual patient, the findings remained unchanged throughout the one-year period. During the second year of therapy, however, tubercle bacilli have reappeared in the sputum of 2 patients whose sputum cultures were negative for *M. tuberculosis* at the end of the first year.

There was a generally close agreement between the results of microscopic and cultural examinations of the sputum and gastric washings.

Isoniazid-Susceptibility in Vitro

Of the 43 patients who were discharging tubercle bacilli at the start of therapy, 11 (25 per cent) discharged strains resistant to 1 γ of isoniazid per ml. of medium at one time or another during the one-year period.

Nine of these 11 patients had far advanced cavitary disease which had shown no significant improvement during isoniazid therapy. In 4 of the 11 patients, the lack of significant improvement resulted in a change in therapy, principally the employment of surgery, at some time during the second or third quarters of the therapeutic year. Seven of the 32 patients who completed the year of therapy were discharging "one microgram"[7] isoniazid-resistant strains of tubercle bacilli at that time; the other 4 patients, infectious at that time, were discharging isoniazid-susceptible strains of bacilli.

Of the 11 "one microgram" drug-resistant strains of tubercle bacilli, 5 required 12 to 50 γ of isoniazid per ml. for inhibition and the remainder were inhibited by 3.2 γ (3 strains) or 6.3 γ per ml. of medium. It should be recalled

[7] Designates cultures of *M. tuberculosis* capable of growing in 1 γ of isoniazid per ml. of medium.

that these determinations of drug-susceptibility were all made in liquid medium. Quantitative microbiologic studies of the tubercle bacilli discharged by the 11 patients have been reported elsewhere by Tompsett (33).

Microbiologic Findings Related to Roentgenographic Findings

There was no particular correlation between the microbiologic and the roentgenographic findings. Six of the 11 patients who discharged tubercle bacilli at twelve months were in the group of 21 patients who showed substantial roentgenographic improvement. It is of interest, however, that all of the 12 patients with cavity closure had sputum and gastric washings negative for *M. tuberculosis* on culture.

There was no obvious correlation between progression of disease and the discharge of drug-resistant bacilli. Only 2 of the 6 patients whose disease was classified as *worse* roentgenographically ever discharged "one microgram" isoniazid-resistant bacilli. Nevertheless, 7 of the 11 who did discharge drug-resistant bacilli were in the group with the roentgenographic classification of *no significant* improvement.

Ability to tolerate medication: All of the patients received the isoniazid without incident throughout the "therapeutic year." In no instance was there any symptomatic or objective evidence of peripheral neuritis or, indeed, any manifestations of drug toxicity whatsoever.

Ultimate outcome: Although the 47 patients were started on isoniazid treatment during the period from November, 1951, to August, 1952, inclusive, only the course of their tuberculosis throughout the first year of chemotherapy is described in the present report. The reason for this is discussed below and has to do with the distorting influences of collapse or excisional therapy on drug evaluations in the later months of the "therapeutic year" and thereafter. Without reference to chemotherapy subsequent to the first year of isoniazid alone, it can be said that 31 patients are living outside of hospitals, 3 are dead (one of nontuberculous causes), and 13 were still receiving hospital treatment for tuberculosis when information was last received. Twelve patients are known to have had some form of collapse or excisional therapy subsequent to the first year of isoniazid therapy.

Additional patients who received isoniazid alone for long periods: Approximately 80 patients, in addition to the 47 patients of the present study, have also received isoniazid alone for periods of nine to eighteen months as follow-up chemotherapy after several months of pyrazinamide-isoniazid. The results observed in these patients, all of whom had pulmonary tuberculosis, form the basis of a separate report (11).[5]

[5] In September, 1953, an additional series of hospitalized patients with pulmonary tuberculosis was started on treatment with isoniazid alone. Six-month observations on the first 6 of these patients reveal that 5 of the 6 are noninfectious and 4 have experienced substantial roentgenographic improvement. The roentgenographic improvement in the other 2 patients has been only slight.

DISCUSSION

In the present study it was observed that 32 of 47 patients with pulmonary tuberculosis whose sole chemotherapy was isoniazid completed one year of such therapy despite periodic review to determine whether other therapy might be used to advantage. Another 3 patients could presumably have remained in the group were it not for the fact that, on transfer to other hospitals, their chemotherapy was changed in conformance with individual local practices. At some time during the year, 29 of the 47 patients were classified as having shown substantial roentgenographic improvement and 6 were classified as roentgenographically worse. Cavity closure (all cavities per patient) occurred in 15 of the 44 patients with cavitary disease and occurred without the employment of collapse or excisional therapy. At the end of the "therapeutic year," tubercle bacilli could be cultured from approximately one-quarter of the original group of 47 patients or from one-third of the group followed for the whole period.

It is believed that these observations on the use of isoniazid alone have considerable significance with respect to both the choice of chemotherapy for the hospitalized tuberculous patient and the maximal usefulness of home-care programs for tuberculosis.

Observations such as the present study are not in reality a prime measurement of the long-term effectiveness of isoniazid. Instead they are more a measurement of the selection judgment exercised by the particular physicians concerned. The selection consists of the separation, during the course of treatment, of the patients requiring other therapy (usually surgical) from the main portion of the patients who require only chemotherapy. Moreover, only elementary comparisons can be made between the present data and those from other studies because of the unlikelihood that any two series of cases would represent comparable pulmonary lesions without pretreatment attempts at "matching." It happens that the proportion of patients with substantial roentgenographic improvement in the present study is approximately the same as was found at forty weeks in the USPHS study for both isoniazid alone and streptomycin-PAS, and is not at much variance from the isoniazid-streptomycin values in that study. This does no more than indicate, however, that the single-drug regimen with isoniazid is in the same general class as the multiple-drug regimens, and that there was no culpable error of clinical judgment in continuing the treatment for so long a period. The principal significance of the present results, therefore, rests in the fact that of this group of patients, whose treatment was begun with isoniazid, the majority were maintained on the original therapy because there developed no compelling indication for a change. It is information of this type which in the long run determines the place of a particular therapy, in this case long-term isoniazid, in the array of all the available chemotherapies. The results represent only one aspect of the evidence at hand concerning the long-term maintenance of isoniazid effectiveness, and it is appropriate that they be considered in the context of the larger body of information on the subject.

Consideration of this subject should be prefaced by expressing the belief that perhaps too much attention has been paid to the isolation of isoniazid-resistant tubercle bacilli, a phenomenon the significance of which is quite unclear, at some neglect of what actually happens to patients treated with the drug. Viewed in the latter terms, there are five types of impressive evidence to the effect that the high activity of isoniazid demonstrable initially is generally maintained for many months and for at least the same length of time and in the same frequency as is the case with streptomycin-PAS. The five types of evidence are: the course of miliary tuberculosis under prolonged isoniazid therapy (7); the absence of meningitis during long-term therapy of miliary or primary tuberculosis (7, 34, 35); the course of pulmonary tuberculosis on prolonged therapy in the U. S. Public Health Service (9*a*, 9*b*)[9] and in the present studies; the incidence of the worsening of the disease during therapy with isoniazid as compared with streptomycin-PAS (USPHS study); and the incidence of the reversal of infectiousness. Not included with the five types but of possible relevance is the *nature* of the instances of roentgenographic worsening in the present study.

The evidence from miliary tuberculosis consists first of the fact that relapse during isoniazid therapy has not been observed, a phenomenon which is in striking contrast to the situation with streptomycin. This was clearly demonstrated in the early studies of isoniazid in miliary tuberculosis (7) and, to the writers' knowledge, no instances to the contrary have since been reported. To be sure, this specific phenomenon pertains only to comparisons of isoniazid with streptomycin and not with streptomycin-PAS. In so far as the latter therapy is concerned, however, in the isoniazid miliary studies (7, 34) there was the additional observation that meningitis did not develop early or late during the isoniazid therapy of miliary tuberculosis. This was in striking contrast to the situation with streptomycin-PAS in which meningitis may make its first appearance even during the later months of therapy. Both of these phenomena (absence

[9] The carefully conducted chemotherapy studies of the British Medical Research Council (17) and the Veterans Administration (18, 27, 28, 29) cannot be used in this comparison, for both groups abandoned the practice of starting patients solely on isoniazid after a six-month period which ended around September 1, 1952. The MRC regimen which had been studied consisted of 200 mg. of isoniazid daily for a ninety-day period. After the ninety-day period, the individual physicians caring for the patients were free to choose whatever chemotherapy they deemed advisable. In the Veterans Administration, a number of the patients who had received isoniazid alone during the six-month period of the operation of the program received other chemotherapy when the regimen of isoniazid alone was abandoned. There has been no report on any long-term results in those who might have been continued on isoniazid alone. The interesting study of chemotherapy in nonhospitalized patients by Robins, Abeles, Chaves, and their associates (45) was likewise not suitable for comparison. In their report, eight-month observations on the use of isoniazid alone are presented, but the investigators emphasize that the nature of the tuberculosis in their patients was appreciably different from that in the USPHS and MRC studies. Moreover, as their studies are concentrated on nonhospitalized patients, the factor of the amount of bed rest maintained by the patients would presumably also be considerably different from that which obtained in the other studies.

of relapse and of late[10] appearance of meningitis) permit only one of two inferences. Either the first few months of isoniazid have such a marked effect upon the population of tubercle bacilli that revival of lesions in lung and brain during the succeeding six to nine months does not occur or isoniazid continues to exert antituberculous activity throughout the entire period. The first inference finds little support from the studies in man and animals (10, 11, 12, 36), for these do not indicate any unprecedented or unique influences of isoniazid upon the histopathology of the tuberculous lesion. It is true, however, that the microbial population within the lesion is reduced to a considerably lower level with isoniazid than with streptomycin-PAS (10, 11, 12). Although the question is obviously far from settled, at the present time the inference that the action of isoniazid is long preserved seems the more reasonable one of the two possibilities.

The evidence available from the roentgenographic studies of pulmonary tuberculosis is of several sorts. In both the U. S. Public Health Service study and the present study, a substantial proportion (50.6 to 66 per cent) of the patients showed substantial roentgenographic improvement at forty weeks. This value of 50.6 per cent in the USPHS study was in good agreement with the corresponding value of 50.4 per cent for streptomycin-PAS and not at too great variance with the value of 59.0 per cent for isoniazid-streptomycin also obtained in the same study.[11]

The most important single piece of evidence on the question of long-term maintenance of isoniazid activity in pulmonary tuberculosis, however, is that provided by the incidence of roentgenographic "worsening" in the U. S. Public Health Service study (9*a*, 9*b*). After nine months of therapy (forty weeks) the values for roentgenographic "worsening" were virtually identical among patients who received *only isoniazid* (7.7 per cent), or *streptomycin-PAS* (6.8 per cent), or *isoniazid with streptomycin* (4.3 per cent).[11] (The corresponding value at the same time period in the present study was not too much greater, namely 5 of 35 patients, or 14 per cent). If the effectiveness of isoniazid were usually or even frequently lost after the first few months of therapy, it would be anticipated that by the tenth month appreciably more instances of disease progression despite therapy would have occurred with isoniazid alone than with streptomycin-PAS.

The values for the sustained reversal of infectiousness as determined by microscopy and culture in both the present study and the USPHS study provide evidence that isoniazid effectiveness is generally maintained for long

[10] The abolition of the *early* appearance of meningitis in isoniazid-treated miliary tuberculosis is not germane to the present argument because it could merely reflect better distribution of the drug rather than long-continued maintenance of effectiveness.

[11] Some notion of the range of the significant limits of the roentgenographic method is afforded by a second USPHS study (37) which was conducted similarly to the first study but included no patients who had had previous chemotherapy. In the later study, the twenty-week value for *substantial roentgenographic improvement* on isoniazid-streptomycin was 69.1 per cent compared with 64.3 per cent for a comparable group on an identical regimen except for the concurrent administration of PAS. In addition, three other regimens, consisting of various dosages and pairings of isoniazid with streptomycin or PAS, yielded values of 61.9, 62.3, and 66.3 per cent.

periods. At forty weeks in the latter study, *M. tuberculosis* could be cultured from 29 per cent of the patients who received isoniazid alone; from 32 per cent of those who received streptomycin-PAS; and from 16 per cent of those on isoniazid-streptomycin therapy. In the present study with isoniazid alone, which included no patients who received collapse or excisional therapy, the corresponding forty-week value was 38 per cent (13 of 35 patients).

Finally, there is a sixth type of information which may be derived from the present study in pulmonary tuberculosis, i.e., the *nature* of the "roentgenographic worsening" under long-term isoniazid therapy. Information of this type is less definite evidence than the five points mentioned above and, indeed, might conceivably be misleading. Nevertheless, the observations are sufficiently provocative to be worthy of mention. In the present study, 5 of the 6 instances of "roentgenographic worsening" consisted of either a slight increase in size of a density or cavity previously present or the first appearance of translucency, and hence, presumably excavation, in a density previously present (plates I to VI, inclusive). With one questionable exception (plate II), there were no instances of the first appearance at any time during therapy of a definite density in an area clearly separated from the original lesion and previously free from demonstrable disease. In a previous experience with streptomycin (38), the first appearance of totally new areas of pulmonary disease during therapy (exclusive of postoperative phenomena) was not uncommon. Whether such an occurrence during isoniazid therapy is in fact as rare as in the present series cannot be established at present. Unfortunately, the specific point is not catalogued in the reports of the USPHS and other large-scale cooperative investigations. If subsequent experience confirms the suspicion evoked by the present study that such development of unequivocally new areas of pulmonary disease[12] during isoniazid therapy is indeed rare, it will establish another type of evidence on this question of the continued maintenance of isoniazid activity.

The discussion up to now can be summarized as follows. A variety of types of observation in treated patients (as distinguished from drug-susceptibility tests *in vitro*) forms an impressive body of evidence that isoniazid generally maintains its activity for long periods (at least nine to twelve months), or at least for as long as does streptomycin-PAS. With the current fashion so strongly in the direction of double- or triple-drug therapy, it is unlikely that any additional information on various aspects of isoniazid alone versus streptomycin-PAS will be forthcoming. *It is important to note, therefore, that on the basis of actual long-term trial there is no evidence that isoniazid loses its effectiveness in pulmonary tuberculosis any sooner or to any greater extent than does streptomycin-PAS.* In this respect the information derived from studies in pulmonary tuberculosis and from miliary tuberculosis has been identical.

[12] In contrast to this situation which appears to exist with pulmonary tuberculosis, the enlargement of cervical or mediastinal *lymph nodes* while miliary or other pulmonary lesions are satisfactorily regressing on isoniazid therapy has been noted by several groups of investigators (7, 45). In all such instances observed by the present investigators, the enlarged lymph nodes have soon regressed satisfactorily without any addition to, or changes in, the regimen of isoniazid alone.

In pulmonary tuberculosis, a form of the infection which does not permit too fine comparisons of drug activity, isoniazid and streptomycin-PAS are thus crudely comparable in terms of both drug effectiveness and its maintenance with the passage of time. It is only natural, therefore, to wonder whether the administration of streptomycin or PAS *along with isoniazid* would result in a significant enhancement, or more importantly, in a prolongation of total antimicrobial activity against *M. tuberculosis* in humans. By *enhancement* of total antimicrobial action is meant the "incapacitation" of more tubercle bacilli from their disease-producing activities than would otherwise be the case.

The objection might be raised that an attempt to make a distinction between the *enhancement* and the *prolongation* of antimicrobial action is pointless, for a particular drug regimen might result in both. Certainly it is conceivable that two or more chemicals administered together might in some specific way produce an enhancement of total antimicrobial action which could also be indefinitely prolonged. It is likewise conceivable that enhancement could be produced for a period, only to be terminated by the escape of the bacilli from drug influence. Prolongation without discernible enhancement is also conceivable.

The point for consideration, therefore, is not whether such phenomenon are credible possibilities but whether with the available drugs and the specific case of isoniazid they actually occur. A corollary issue is how much antimicrobial action is necessary or useful once the initial maximal effects have been produced. It is conceivable that the major part of what can be accomplished in terms of incapacitating tubercle bacilli is actually accomplished within the initial weeks of therapy and that the chief useful purpose served by therapy thereafter is to ensure the continued "physiologic imprisonment" of the surviving microorganisms. If this were the case, the situation with respect to isoniazid-streptomycin versus isoniazid alone might be considerably different in the early and in the late phases of therapy.[13]

Unfortunately, this question of enhancement by the use of isoniazid-streptomycin or isoniazid-PAS is one which probably cannot be convincingly answered by direct studies of the behavior of tuberculous lesions in patients. The response of miliary tuberculosis to isoniazid is so uniform and sustained that the effects of a second drug would not be likely to be measurable. In pulmonary tuberculosis, a difference in the effectiveness of two regimens, *both of which contain isoniazid*, must be quite marked for it to be demonstrable by the roentgenographic method.[11] Nevertheless, it is conceivable that careful clinical investigations could be organized which might provide some information on the question. For example, a large-scale cooperative study could be made in which the speed of defervescence in miliary tuberculosis could be used in a comparison of isoniazid alone with isoniazid-streptomycin or isoniazid-PAS. Likewise, a cooperative study limited to comparably fresh and extensive pneumonic lesions might be

[13] After this was written, the present writers became acquainted with the unpublished experiences of Drs. Middlebrook and Dressler at the National Jewish Hospital in Denver (44). These investigators administer relatively large doses of isoniazid and streptomycin during the first few weeks of therapy and continue thereafter with isoniazid alone.

attempted although the administrative problems in obtaining meaningful numbers of suitable cases would be very real.

In the absence of such pointed studies, judgment must be made on the basis of the available data from observations in man and in experimental animals. Using three sets of criteria, roentgenographic evaluation in humans, the incidence of reversal of infectiousness in humans, and microbial enumeration studies in mice, it has not been possible to establish with absolute certainty that isoniazid-streptomycin is superior to isoniazid alone. Nevertheless, in all three types of study, the actual values determined were all slightly in favor of isoniazid-streptomycin. In the USPHS study (9*a*, 9*b*),[14] the value for substantial roentgenographic improvement was 7 per cent higher for isoniazid-streptomycin than for isoniazid alone at twelve weeks and 9 per cent higher (50.6 and 59.0 per cent) at forty weeks, when the study was terminated. Likewise, the proportion of patients discharging culturable tubercle bacilli after forty weeks of treatment was approximately 10 per cent lower on isoniazid-streptomycin than on isoniazid alone (approximately 15 and 25 per cent, respectively).

In the microbial population studies in mice (10, 11, 12) it was not possible to demonstrate a significant difference between isoniazid-streptomycin and isoniazid alone in terms of reduction of the population of *M. tuberculosis* in lung and spleen. Here again, however, the actual values (before statistical analysis) were always slightly in favor of isoniazid-streptomycin.

With respect to isoniazid-PAS versus isoniazid alone, it should be emphasized that there has been no investigation in which these two regimens of chemotherapy have been measured against each other in comparable groups of patients studied concurrently. Consequently the only information available is that which might be derived by comparing the results on isoniazid alone in the early studies with the results on isoniazid-PAS in subsequent studies. As might be anticipated, such a comparison is not very satisfactory, for the results with isoniazid-streptomycin (the one drug-pairing common to all of the studies) were considerably different when studied by the same investigators at different time periods.

For example, substantial roentgenographic clearing at twenty weeks[15] occurred in 33.2 per cent on isoniazid alone in USPHS study I (9*a*, 9*b*) compared with 62 per cent for isoniazid-PAS in USPHS study II (37). At first glance this 29 per cent difference seems impressive. On further scrutiny, however, it may be seen that two isoniazid-streptomycin regimens in USPHS study II were

[14] It was not possible to make accurate comparisons on these points with the data in the MRC study. For, in the MRC study, the values are recorded in months rather than weeks and, in the study in which isoniazid alone was compared with isoniazid-streptomycin (17), the streptomycin was given daily (1 gm.) instead of twice weekly as in the USPHS study. With due allowance for these differences in methodology, the three-month values for substantial roentgenographic improvement, i.e., two-plus and three-plus, in the MRC study were 26 per cent for isoniazid alone and 29 per cent for isoniazid-streptomycin. The values for culturable tubercle bacilli were 55 per cent for isoniazid alone and 24 per cent for isoniazid with daily streptomycin.

[15] In this and all subsequent comparisons, the time period chosen represents the longest one for which the data for comparison are available.

likewise 25 and 28 per cent superior in terms of substantial roentgenographic improvement to the isoniazid-streptomycin regimen in USPHS study I. A similar situation exists with respect to the two British MRC studies. A comparison of the value (at three months) for substantial roentgenographic improvement on isoniazid alone in MRC study I (17) (26 per cent) with that obtained with isoniazid-PAS (39 per cent) in study II (17*d*) yields a 13 per cent difference in favor of isoniazid-PAS. An even greater difference exists, however, i.e., 20 per cent, between the corresponding roentgenographic results on isoniazid-streptomycin in MRC study I (29 per cent) and the results with the same two drugs in MRC study II (49 per cent).

These substantial differences between studies I and II with respect to roentgenographic improvement on isoniazid-streptomycin, which were found on both sides of the Atlantic,[16] presumably reflect the presence of a greater number of reversible lesions in the later studies. The bacteriologic data should also be affected by such a phenomenon, but from the preliminary observations, at least, this does not seem to be the case. Hence, the nominal superiority of isoniazid-PAS over isoniazid alone in terms of the reversal of infectiousness which can be derived from comparisons of the older with the newer studies may have substance. For example, in the British studies, tubercle bacilli could be cultured at three months from 24 per cent of the patients who received isoniazid-streptomycin in study I, and from 18 per cent of those who received the same drugs in study II. Hence, the isolation of tubercle bacilli from only 17 per cent of the patients who received isoniazid-PAS in MRC study II stands out prominently in comparison with the corresponding 55 per cent value for isoniazid alone in MRC study I (17*d*). In the American studies, the corresponding values at sixteen weeks for patients on isoniazid-streptomycin were 21.1 per cent in USPHS study I and 18.1 and 20.9 per cent in USPHS study II. At the same time in the latter study, *M. tuberculosis* was cultured from 8.6 and 12.9 per cent of two groups who received isoniazid-PAS and from 14.9 per cent of a third group who received isoniazid-PAS and streptomycin. This range of 8.6 to 14.9 per cent is to be compared with a sixteen-week value of 34.6 per cent for isoniazid alone in USPHS study I.

Unfortunately it is not possible to determine how much of this apparent 20 to 38 per cent superiority of isoniazid-PAS over isoniazid in terms of reversal of infectiousness is attributable to the PAS. For, in addition to the lack of evi-

[16] It should be stressed emphatically that the data derived from the comparative analyses used in this Discussion do not in any way represent "claims" of these investigators which are here being subjected to criticism. On the contrary, the individual reports from both investigations have been noteworthy for their sharply defined limitation to the specific question or questions under study and for their cautions concerning the degree of significance of the various sets of data. Moreover, both investigations have been specifically designed to permit comparisons between the first and the second studies by selection of similar subgroups for separate analysis (e.g., all patients with noncavitary disease known to be of less than three months' duration). Hence, it is anticipated that comparisons of the material from appropriately chosen subgroups from Studies I and II of both investigations will be forthcoming and should shed considerable light on these questions.

dence that the isoniazid-PAS regimen is superior in terms of roentgenographic change (as outlined above), there are the observations of Fruhlinger and Bala (39), who state that as much as 20 per cent of the negative sputum cultures for tubercle bacilli obtained during PAS therapy represent artefacts. Ideally, this highly important question of whether PAS along with isoniazid does materially increase isoniazid activity should be settled by a comparative study of isoniazid alone and isoniazid-PAS in treated patients. Failing that, the matter will have to be decided by inferences drawn from comparisons with isoniazid-streptomycin or by additional analyses of comparable subgroups in the USPHS and MRC material.

Finally, in this connection, it would seem that, if PAS along with isoniazid results in increased antimicrobial activity analogous to that of PAS with streptomycin, or the even more striking pyrazinamide-isoniazid phenomenon (46, 47), some indication of the superiority of isoniazid-PAS over isoniazid alone should eventually appear in the roentgenographic observations.

In the microbial population studies in mice (10, 11, 12) the addition of PAS does not enhance the activity of isoniazid. In contrast, in the mouse as in man, the addition of PAS definitely enhances the activity of streptomycin.

In essence the available evidence fails to show that isoniazid-streptomycin enjoys a substantial advantage over isoniazid alone (or over streptomycin-PAS in the clinical studies) in terms of greater effectiveness during the early weeks of therapy. By the same token there are indications that isoniazid-streptomycin does have some advantage, however slight. At present, therefore, when the clinician is faced with a situation in which maximal antimicrobial therapy would be especially desirable, e.g., acute tuberculous pneumonia, certain cases of meningitis, he must weigh the apparently slight advantages of isoniazid-streptomycin against the disadvantages, largely theoretic, which might be associated with such a regimen.

The use of PAS with isoniazid presents from the disadvantage standpoint essentially the same situation as is the case with streptomycin (*see* below). From the advantage standpoint, however, the situation with respect to isoniazid-PAS is less clear. Of three criteria, roentgenographic change, microbial enumeration in mice, and reversal of infectiousness, only the third seems to indicate a superiority of isoniazid-PAS over isoniazid alone and the true meaning of even this finding is subject to some question. The question of the possible disadvantages of isoniazid-streptomyin or isoniazid-PAS will be discussed after consideration of the possible advantages of these pairings with respect to *prolongation* of antimicrobial action.

The question of *prolongation* of antimicrobial activity is perhaps of much greater importance than the one of enhancement. For prolongation would have a bearing in every case and not just in the specialized circumstances in which a significant enhancement would be most desirable. Indeed, if effective antimicrobial action could be supplied uniformly for an unlimited period, it is conceivable that important benefits would be demonstrable in those patients (now approximately one of three) with pulmonary tuberculosis who are not satisfactorily benefited by any of the present forms of chemotherapy.

Thus, this perplexing problem of whether to use streptomycin or PAS along with isoniazid becomes one of truly major importance, only if it can be shown that their use as a pair or trio actually does *prolong* the period of antimicrobial effectiveness. The principal question at issue, therefore, is whether some pairing of isoniazid with streptomycin or PAS or all three together will result in maintenance of antimicrobial action in a larger proportion of a group of pulmonary cases and for a longer period than is presently the case with isoniazid, on the one hand, or streptomycin-PAS, on the other.

Unfortunately, this is a type of question which is even less likely to be answerable from studies in man than is the question of enhancement. For in the last analysis the problem of the continued presence or the disappearance of drug effectiveness can only be measured in terms of relapse during therapy. To all intents and purposes this means roentgenographic worsening or the reappearance of tubercle bacilli after their prolonged absence from the sputum. In miliary tuberculosis, relapse has not been observed during or after appropriate treatment with isoniazid alone and occurs only rarely, if at all, with streptomycin-PAS (in contrast to the situation with streptomycin alone). Likewise, in pulmonary tuberculosis, from the present study with long-term isoniazid alone and the USPHS study of this and other chemotherapies, it is clear that relapse as reflected by "roentgenographic worsening" during treatment is infrequent. Moreover, in the tenth month of therapy in the Public Health Service study, relapse, i.e., "roentgenographic worsening," appeared in essentially the same incidence with isoniazid alone, isoniazid-streptomycin, and streptomycin-PAS. Hence, within a nine- to ten-month period it was not possible to detect any difference among the three regimens with respect to the prolonged maintenance of drug action. If it has not been possible to demonstrate any such difference within the first ten months of therapy, it will be virtually impossible to do it thereafter. The reason for this has to do with the important question of the use of collapse or excisional therapy in patients whose pulmonary tuberculosis is under study for the degree of response to antimicrobial drugs.

Actually the necessity for performing collapse or excisional therapy (including pneumoperitoneum) in a substantial minority of patients with pulmonary tuberculosis poses a great dilemma in the interpretation of the effects of antimicrobial therapy after the sixth month of most therapeutic trials. One procedure is that which has been followed in the USPHS and Veterans Administration studies in which drug evaluation is continued in all patients even though excision or collapse therapy has been performed. The justification for this practice is that by and large the percentage of patients who receive collapse or excisional therapy tends to be the same throughout the various experimental groups; hence, the distorting effects of the practice may be disregarded. Indeed during the first six months or so of most therapeutic studies it is not likely that collapse or excision are distorting factors. For, with due allowance for individual differences in local practice, it is customary to permit a period of approximately six months of chemotherapy to elapse before making any final judgments about additional forms of therapy.

Once past the sixth month, however, the situation becomes considerably

changed. From this point on, the patients whose lesions are not being completely "arrested" by chemotherapy become increasingly easy to identify and are properly subjected to the additional procedures. Irrespective of the type of chemotherapy, once the tuberculous lesions have been collapsed or actually excised, the chances are obviously excellent that tubercle bacilli will no longer be excreted in the sputum. Hence, it is obviously a distortion to continue such patients in an experimental series in which the status of the sputum with respect to tubercle bacilli is used as an index of drug activity.

The opposite practice (followed in the present study) of eliminating patients from the study series once their lesions have been collapsed or excised is likewise a distortion. For the patients available for microbiologic studies between the eighth and twelfth months of chemotherapy represent a group composed of those who either require no collapse or excisional therapy or in whom such therapy is technically inadvisable.

Thus, either of the two practices introduces a distortion which becomes increasingly operative with the passage of time, notably during the last half or third of the first year of chemotherapy. Moreover, it would not be ethically justifiable to withhold or postpone collapse or excisional therapy in patients obviously needing such therapy merely for the purpose of evaluating antimicrobial drugs. Consequently, the only reasonable solution of this dilemma which suggests itself is simply to recognize that *it is not really satisfactory to use cultural studies as an index of drug effectiveness in pulmonary tuberculosis past a certain point in time*. The time sequences involved are such that in any group of patients with pulmonary tuberculosis the factor of collapse or excision is negligible during the first six months of study; becomes a disturbing factor between the sixth and the ninth month; and from the ninth month on represents such a distorting factor that inferences based on reversal of infectiousness between groups on two different regimens of chemotherapy are almost certainly no longer meaningful.

Thus, about the only way in which the question of the prolongation of effective drug action could be measured past the eighth or ninth month of therapy would be by measurement of progression of disease and return of infectiousness during therapy among the survivors of the various groups which receive no collapse or excisional therapy.

In the absence of such information and within the framework of the available time periods, i.e., one year, there is no evidence that any of the three regimens of isoniazid alone, isoniazid-streptomycin, or streptomycin-PAS is superior to the other two in terms of prolongation of effectiveness.

The situation with respect to the use of all three drugs together is essentially the same as that presented by isoniazid-streptomycin or isoniazid-PAS. In the microbial population studies in mice, there was no evidence that the administration of both streptomycin and PAS along with isoniazid leads to any enhancement of antimicrobial action over that produced by isoniazid alone (10, 11, 12). Likewise, in the USPHS studies in humans (37), there was no demonstrable advantage of the triple-drug regimen over the various two-drug regimens in

terms of substantial roentgenographic improvement at twenty weeks or absence of infectiousness at sixteen weeks. One aspect of the question of triple-drug therapy has not yet been defined. There is no information whether the isoniazid-streptomycin-PAS regimen used over a long period, for example two years, would provide more satisfactory antimicrobial action throughout the entire period than would be the case with isoniazid alone for one of the years and streptomycin-PAS for the other.

As mentioned previously, the disadvantage which might possibly accrue to a patient from the use of isoniazid and streptomycin together is based largely on theoretic considerations. The argument runs about like this.

> Pulmonary tuberculosis is a chronic disease for which an infected person might have to have several long periods of chemotherapy throughout his lifetime. Tubercle bacilli resistant to either isoniazid, streptomycin, or PAS eventually appear and apparently increase in numbers in any patient who continues to discharge bacilli despite continued treatment with one of these drugs. It is clear that streptomycin-resistant tubercle bacilli maintain full pathogenicity and are not affected by streptomycin *in vivo* (31). It is likewise clear that some isoniazid-resistant tubercle bacilli are also changed in ways other than their drug susceptibility so that their pathogenicity is altered (40, 41, 42). Nevertheless, there is solid evidence that certain isoniazid-resistant tubercle bacilli have the capacity for continued multiplication within the isoniazid-treated mouse (33, 43). In this situation they are clearly pathogenic and may also be pathogenic for man. In the face of this evidence, therefore, it would be foolhardy to assume the position that certain isoniazid-resistant bacilli might not be disadvantageous to the patient. There has been less intensive study of PAS from this standpoint, but there is no reason to believe that its situation is materially different from that of streptomycin (49). Tubercle bacilli resistant to both isoniazid and streptomycin, or to streptomycin and presumably PAS, can be derived from patients receiving either pair of drugs (9c).
>
> The physician does not usually wish to use streptomycin alone, for the time limits of its period of effectiveness have been quite well established. By the same token, PAS is seldom used alone, for it is a relatively weak agent by itself even though it has considerable usefulness when administered with streptomycin. When the physician chooses to use either streptomycin or PAS along with isoniazid, however, his patient is at risk, that whichever adjuvant is used, i.e. either streptomycin or PAS, it may be compromised for future usefulness in that patient. It is not always appreciated that this theoretic disadvantage applies with equal force to PAS as well as to streptomycin and that a patient whose tubercle bacilli are PAS-resistant can look forward to nothing but short-term benefit if it be subsequently necessary to use streptomycin.
>
> In essence, the argument is that anything which tends to compromise the value of either member of the streptomycin-PAS pairing negates to that extent the future usefulness of streptomycin.

It should be emphasized that the present writers are by no means convinced that the above argument has widespread validity. Nevertheless, the data bearing on the point are too few to make a convincing denial of the argument at present.

* * *

The foregoing considerations regarding multiple-[17] and single-drug therapy leave the physician in a position of some uncertainty, but also with considerable latitude of choice, in planning the treatment of a patient with pulmonary tuberculosis. It would be surprising if, as experience accumulates, a statistically valid basis for individualization of treatment according to specific situations and types of disease did not emerge. Until more statistical information is available on results with various regimens, not only in control series of unselected cases of pulmonary tuberculosis, but also in selected types, no categoric statements are justified concerning what is the therapy of choice.

The regimen for starting treatment which is still most frequently recommended in current literature as the therapy of choice is streptomycin-PAS. As the clinical evidence indicates, however, that this regimen is neither of greater nor of more prolonged effectiveness than isoniazid singly, the choice seems at best a doubtful one. Particularly is this true if the dosages of streptomycin and of PAS are those usually employed in the United States, i.e., streptomycin, 1 gm. twice weekly, and PAS, 12 gm. daily. The higher doses of PAS customary in Great Britain, and the more continuous (daily) administration of streptomycin, may give a slight edge to streptomycin-PAS over isoniazid. Whether employed in the more intensive or in the less intensive manner, streptomycin-PAS has material disadvantages in contrast to isoniazid with respect to cost, ease of administration, and relative freedom from major as well as minor toxicity.

It seems more reasonable, therefore, to administer isoniazid as the first chemotherapy, especially in situations in which it is estimated that a maximal antimicrobial action is not essential. Pulmonary tuberculosis of only minimal extent might well be considered to fall usually in this category. In contrast, advanced tuberculous pneumonia with massive caseation might be considered to require the maximal degree of antimicrobial effect which can possibly be attained. For this purpose the supplementation of isoniazid with either streptomycin, PAS, or both drugs would seem desirable because of the evidence that some degree, at least, of enhancement may be so effected. Between this and the

[17] The terms *multiple therapy* and *single-drug therapy* are used in this discussion solely for ease in presentation. Strictly speaking, this question of the choice of chemotherapy cannot be properly considered in terms of multiple drugs per se versus single drugs per se. Instead, it must be recognized that, in tuberculosis as in other infections, a certain relatively few drug pairings result in significant enhancement of total antimicrobial activity, whereas most other multiple-drug regimens do not. The latter phenonemon is usually the case even though the individual drugs in the multiple-drug regimens may be quite powerful in themselves. In nontuberculous infections the outstanding examples of drug pairings which lead to enhancement of antimicrobial action, e.g., true synergism, are the dihydrostreptomycin-penicillin treatment of enterococcal endocarditis (48) and the primaquine-chloroquine treatment of malaria (50 *a* and *b*). In tuberculosis, only two drug pairings have thus far been definitely shown to result in significant antimicrobial enhancement in animals, streptomycin-PAS and pyrazinamide-isoniazid. It appears, therefore, that however little may be known concerning the mechanics of these effective drug pairings, the resulting phenomena bear the aspect of true specificity and not that of a mere sum of any two powerful drugs. Hence, it seems appropriate, in so far as possible, to consider these matters in terms of particular drugs and particular therapeutic situations.

other extreme of an early and almost completely reversible minimal lesion lies the entire spectrum of mixed types with variable proportions of reversible and irreversible elements. The assumption that the therapy of choice for all of the different patterns of pulmonary tuberculosis, classifiable as moderately and far advanced, is necessarily a multiple drug regimen seems as doubtful as that such multiple therapy is usually necessary in minimal tuberculosis.

Patients with unilateral cavitary disease of unknown duration and uncertain reversibility, for whom eventual excisional surgery must be considered, may well be treated initially with isoniazid. A three- to six-month trial of such therapy may often result in making it unnecessary to use other drugs or, indeed, to perform any operation. If clinical arrest is not attained and surgery does become necessary, the pre- and postoperative periods can be additionally "protected" by streptomycin, which has in this plan not been compromised by prior administration. Moreover, streptomycin may now be depended upon for prolonged postoperative therapy because, by the initial administration only of isoniazid, there is still available the equally uncompromised PAS for a companion drug to streptomycin. Isoniazid may reasonably be continued as a third drug, if desired, as there is little, if any, evidence that its prior administration for three to six months necessarily destroys its value for combined treatment any more than for single-drug therapy.

The examples cited above are but illustrations of possible and reasonable selections of therapy for certain types of clinical problems. Hitherto the comparative-regimen studies in pulmonary tuberculosis have been planned to obtain an inclusive answer to the question of which of currently available drug regimens is best for pulmonary tuberculosis. Now that the number of effective agents available has increased, it will become necessary to subdivide the case material, and such analyses are already in progress[16]. It might be objected that it must first be determined what regimen is most successful on an over-all statistical basis, without selection or subdivision of case material according to extent and type of disease. When the difference is as great, however, in terms of inconvenience and of drug intolerance as it is between isoniazid singly and triple-drug therapy, or isoniazid and isoniazid-PAS, or isoniazid and isoniazid-streptomycin, even an over-all statistical superiority of multiple-drug therapy would not necessarily make its *indiscriminate use* the best practice while awaiting the collection of more particularized data.

At the present time the demonstrated differences between the various regimens of isoniazid, streptomycin-PAS, isoniazid-streptomycin, isoniazid-PAS, and isoniazid-streptomycin-PAS are not sufficiently great to indicate that any one of them is indisputably the therapy of choice for all varieties of clinical tuberculosis, or even for all varieties of pulmonary tuberculosis. On the basis of available evidence, certain advantages of multiple-drug therapy appear to make this advisable in situations in which the desirability of an immediate maximal drug action is evident. Multiple-drug therapy has certain disadvantages in addition to the question of immediate practicability which make it eminently desirable to avoid the administration of more than one drug whenever this is

possible. Among these disadvantages is the important consideration that each drug administered may be compromised, at least to some extent, with respect to its future usefulness. As there is no evidence that any combination of these three drugs provides an eradicative type of therapy, and as many factors unrelated to drug treatment may cause relapse, this disadvantage, in the long view, assumes a paramount importance. Economy of therapy is desirable, not only in the monetary sense but from the strictly therapeutic standpoint of holding something in reserve. Although no longer so obvious as when streptomycin was the sole effective agent at hand, and although perhaps not of such immediate importance as it was, this principle of saving something for eventualities is still a practical consideration. Although it may be reasonable to expect further additions to the growing list of effective agents, it is improvident to depend entirely on such hope.

The other agents presently available or under study, such as viomycin and pyrazinamide, are not to be forgotten either. These are not, however, independently satisfactory agents for prolonged therapy, and viomycin is clearly a less effective agent in safe dosage than either isoniazid or streptomycin. Pyrazinamide, however, despite its demonstrated hepatic toxicity and its short-lived duration of activity when administered alone, must still be considered as potentially a most valuable agent. In combination with isoniazid, it provides probably the most effective therapy known and one which, moreover, is the simplest multiple therapy to administer and has the least unpleasant minor side effects (11, 46, 47). If the problem of hepatic damage proves avoidable without loss of therapeutic effect, pyrazinamide-isoniazid may indeed emerge as the therapy of choice for the severe and potentially most serious clinical situations. For the present, however, its use must be confined to investigative programs.

The four regimens composed of various combinations of isoniazid, streptomycin and PAS, plus isoniazid as single therapy, thus comprise the choices available for general use. Single-drug therapy with either streptomycin or PAS has little to commend it. The accumulated experience of some years indicates that these two agents are best used in combination with each other, or perhaps each with some third drug. The choice between the five regimens can be approached with rational discrimination appropriate to particular clinical circumstances even if definitive evaluations are still pending. Probably of even greater importance than the actual selection of a drug regimen are the continuity and duration of its administration. Finally, no drug therapy in tuberculosis can be employed to optimal advantage without frequent periodic reviews of the situation with reference to the timely addition or substitution of other measures. These include not only rest therapy, collapse procedures and excisional surgery, all of which are of proved value for particular indications, but also alterations in the drug therapy itself.

* * *

Another aspect of the present study with isoniazid alone which is of interest has to do with the use of chemotherapy in home-care programs. At the outset it may be noted that the variety of therapy possible in a home-care program

obviously depends upon: the proximity of the patient to the physician or hospital; the frequency of nursing visiting which is feasible; the enthusiasm and continued cooperation of the patient; and, in some cases, the patient's willingness or capability to learn minor technical procedures.

In certain ideal circumstances, any regimen of therapy available in a hospital can be made available in the home. In many such situations, home care represents not an improvisation but merely a matter of individual preference. On a wider basis, home-care programs utilizing streptomycin-PAS have been in operation for some years, principally in large urban areas with a case load disproportionate to the inpatient hospital facilities. When isoniazid was first introduced, it was quickly incorporated into such home-care programs and gave them a wider choice of chemotherapies, as it did in hospital practice.

It is no discredit to these "metropolitan type" programs, which can utilize multiple-drug regimens, to state that the broad field of usefulness of home-care programs does not lie primarily in the highly industrialized urban centers. On the contrary, the principal usefulness of home-care programs would be in areas of low technologic development in which patient cooperation can be obtained but where hospital facilities are inadequate or nonexistent. To all intents and purposes, a therapy for widespread usefulness in such a situation must be self-administered, which means an oral preparation. The therapy must also be sufficiently well-tolerated so that the patient will continue to take the medicine even though he is unsupervised for several days or weeks at a time. The digestive disturbances produced by PAS constitute a serious handicap to its usefulness in such a program. This would particularly be the case when all communication is through nonprofessional interpreters and hence the understanding and enthusiasm of the patient might be less than complete. Indeed, in most circumstances, it would be inadvisable to even attempt to use PAS lest the patient should revolt against all medication.

In the opinion of the writers, the significance of the introduction of isoniazid does not rest primarily on the fact that it represents a powerful tuberculosis chemotherapy, for such powerful therapies were already in existence. In the long run, the significance of the isoniazid development rests on the fact that for the first time it has become technically feasible and rewarding to deliver a significant degree of antituberculous therapy and periodic professional scrutiny to tuberculous patients remote from highly organized hospital facilities.

From the results of the present study with isoniazid alone, it may be seen that a substantial minority of patients with pulmonary tuberculosis would remain infectious and show no roentgenographic evidence of improvement if their only therapy consisted of rest at home on isoniazid. It is clear, therefore, that for widespread, largely unsupervised use in a population with a high prevalence of active tuberculosis, isoniazid alone would benefit some, but would result in no benefit and might even produce harm to many others. It is equally clear, however, that, when such a group of patients is periodically subjected to medical review so that those requiring collapse or excisional therapy can be transported to facilities where they may receive additional treatment, the use of isoniazid alone represents a simple and surprisingly satisfactory regimen of

chemotherapy. Moreover, from the experience in the present series it appears that the patients requiring the additional therapy will not represent a majority. It further appears that the critical period with respect to selection for other therapy is between the fourth and eighth months after the start of therapy. Thus, although there are definite limitations to the use of isoniazid alone in a home-care program, these limitations can be properly managed if the program is intimately associated with facilities for continued medical review and the proper introduction of alternative therapies.

It is to be emphasized that the results obtained from the use of isoniazid alone in the present study were not obtained in unsupervised patients, but rather in patients maintained under good hospital conditions. There is as yet no way of knowing, therefore, whether comparable results could be obtained in patients attempting to receive a greater or lesser amount of bed care at home. Nevertheless, the results of the present study have obvious relevance to medical care programs in areas hopelessly inadequate in hospital facilities in the present or in the foreseeable future.

* * *

The treatment of pulmonary tuberculosis, although vastly more effective as the result of recent progress and new developments, is not thereby made any simpler. On the contrary, the expanded choice of available treatments makes the clinical management of the disease considerably more complicated. The problems of defining specific indications for various measures depend for their solution on continued and more selective investigative programs. The problems of formulating the most effective and least harmful programs of therapeutic management for individual patients depend on clinical judgment and the considered application of all currently available information. This is a formidable task in this present period of rapid change which permits no simple inclusive formulation of tuberculosis treatment. Nevertheless, a rationale can be developed appropriate to the particular clinical problem which need not be so arbitrary as to always include multiple drugs and which should provide for an orderly sequence of a variety of therapeutic measures to be applied as they may be needed.

SUMMARY

Forty-seven patients with pulmonary tuberculosis, moderately advanced or far advanced in extent, were started on isoniazid therapy with the expectation that it would be continued for at least one year. Any patient whose therapy was altered in any way during the year, either by a change in drugs or by the use of collapse or excisional therapy, was eliminated from the study at that time.

Thirty-two of the 47 patients completed one year of such therapy, and another 3 could presumably have remained in the group were it not for the fact that on transfer to other hospitals their chemotherapy was changed in conformance with local practice. At some time during the year, 29 of the 47 patients were classified as having showed substantial roentgenographic improvement and 6 were classified as roentgenographically worse. In no instance did the roentgenographic worsening represent a major spread of the disease. Cavity closure

(all cavities per patient) occurred in 15 of the 44 patients with cavitary disease and occurred without the employment of collapse or excisional therapy. At the end of the therapeutic year, tubercle bacilli could be cultured from approximately one-quarter of the original group of 47 patients, or one-third of the group followed for the whole period.

It is believed that these observations on the use of isoniazid alone have significance with respect to both the choice of chemotherapy of the hospitalized tuberculous patient and the maximal usefulness of home-care programs for tuberculosis. It is further believed that the results suggest the necessity for a reappraisal of the current practice of multiple-drug therapy irrespective of the status of the tuberculous lesion or the particular drugs concerned.

SUMARIO

La Evolución de la Tuberculosis Pulmonar Durante La Terapéutica a Largo Plazo con una Sola Droga (Isoniacida)

En 47 enfermos de tuberculosis pulmonar, moderadamente o muy avanzada en su difusión, se inició la isoniacidoterapia con la idea de continuarla a lo menos un año. Todo enfermo cuya terapéutica fué modificada en cualquier forma durante el año, ya por un cambio de droga o por el uso del colapso o la excisión en el tratamiento, fué eliminado del estudio en dicha ocasión.

Treinta y dos de los 47 enfermos pudieron terminar el año de la terapéutica en cuestión y otros 3 hubieran podido ser agregado presuntamente al grupo, de no haber sido porque, al trasladarlos a otros hospitales, les cambiaron empíricamente la quimitoterapia de conformidad con el régimen local. En alguna ocasión durante el año, se clasificó a 29 de los 47 enfermos como mostrando decidida mejoría roentgenográfica y a 6 se les clasificó como empeorados radiográficamente. En ningún caso representó el empeoramiento radiográfico difusión importante de la enfermedad. Observóse cierre de cavernas (de todas las presentes en el enfermo) en 15 de los 44 sujetos que tenían affección cavitaria, teniendo esto lugar sin el empleo del colapso o de la excisión en el tratamiento. Al cabo del año de terapéutica, se pudieron cultivar bacilos tuberculosos aproximadamente de la cuarta parte del grupo primitivo de 47 enfermos, o sea la tercera parte del grupo mantenido en observación todo el período de tiempo. Parece que estas observaciones relativas al uso de la isoniacida por sí sola poseen importancia con respecto tanto a la selección de la quimioterapia para el tuberculoso hospitalizado como a la factibilidad de la asistencia domiciliaria en la tuberculosis. Parece además que los resultados sugieren la necesidad de justipreciar de nuevo la práctica corriente de implantar una politarmacoterapia, haciendo caso omiso del estado de la lesión tuberculosa o de las drogas dadas usadas.

RESUME

Le cours de la tuberculose pulmonaire durant le traitement de longue durée avec un médicament unique (isoniazide)

Chez quarante-sept malades atteints de tuberculose pulmonaire, de degré avancé ou modéré, le traitement par l'isoniazide a été institué avec l'espoir de le poursu vre pendant au moins une année. Tout malade, chez lequel le traitement était modifié d'une façon quelconque au cours de l'année, soit par un changement dans le médicament soit par une thérapeutique de collapsus ou de résection, a été éliminé de l'étude à partir de ce moment.

Chez 32 malades sur 47 le traitement d'un an a pu être réalisé et 3 autres auraient pu probablement être ajoutés à ce groupe si, à l'occasion de leur transfert dans un autre hôpital la chimiothérapie appliquée n'avait été changée empiriquement et conformément aux habitudes locales. A un certain moment au cours de l'année, parmi les 47 malades, 29 ont

été classifiés substantiellement améliorés du point de vue radiologique et 6 aggravés. En aucun cas, l'aggravation observée sur les clichés n'a représenté une extension importante de l'affection. La fermeture des cavernes (toutes les cavernes dans chacun des cas) s'est produite chez 15 des malades sur 44 atteints de lésions cavitaires; ceci sans que le traitement par collapsus ou la résection ait été appliqué. A la fin de cette cure d'une année le bacille tuberculeux pouvait être obtenu en cultures chez environ un quart du groupe original de 47 malades, soit un tiers du groupe suivi pendant la période tout entière. Les auteurs estiment que ces observations sur l'emploi exclusif de l'isoniazide sont significatives à l'égard du choix de la chimiothérapie chez le tuberculeux hospitalisé, et de la possibilité de réaliser les programmes de soins à domicile (Home-care) dans la tuberculose. Ils considèrent également que ces résultats suggèrent la nécessité d'une ré-évaluation de la pratique courante de la thérapeutique par des médications multiples, quel que soit l'état de la lésion tuberculeuse ou les médicaments particuliers concernés.

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PLATES

PLATE I

Case 1 (E. C.)

Designated as roentgenographically worse during isoniazid therapy.

The patient was a 37-year-old woman whose isoniazid therapy was started on April 16, 1952, for the lesion visible in the right second anterior interspace (left). *M. tuberculosis* was cultured from sputum during the early weeks of therapy but all subsequent cultures of sputum and gastric washings were negative for tubercle bacilli. After eight months of treatment (center), the density had contracted but after nine months (right), an annular shadow interpreted as a cavity was visible. Tomography revealed a similar annular shadow. During the succeeding three months roentgenographic findings remained unchanged and the lesion was resected. Examination of the specimen revealed a healing tuberculous lesion. The cavity was not demonstrated nor was there macroscopic evidence of caseation.

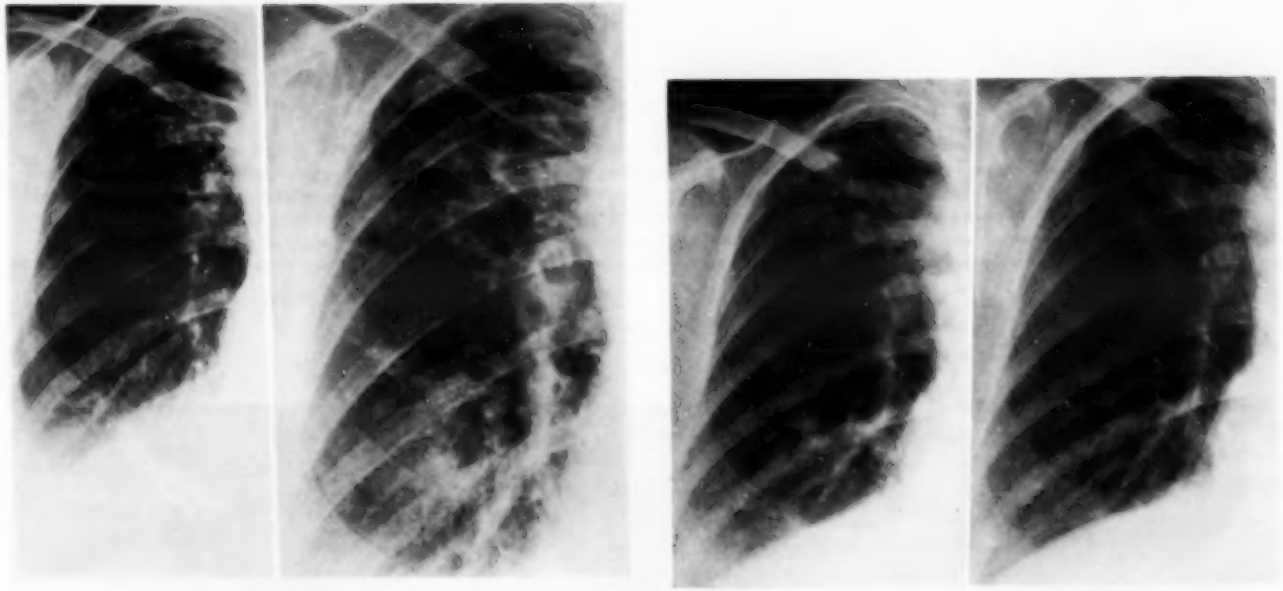


PLATE II

Case 2 (E. R.)

Designated as roentgenographically worse during isoniazid therapy.

A 51-year-old woman whose isoniazid therapy was started on June 7, 1952, for bilateral tuberculosis of the upper lobes. No roentgenographic change was demonstrable between the time of the film at the start of treatment and one obtained seven months later in January, 1953 (left). One week thereafter the patient developed a fever of 104°F., rales could be heard for the first time over the right lower lobe, the leukocyte count was 12,700 per cu. mm. with 50 per cent neutrophils, and a heavy growth of pneumococcus was cultured from the sputum. A roentgenogram (left center) revealed a considerable infiltration in the right lower lung. On penicillin therapy the temperature fell to near normal values but on the seventh day moderate fever recurred and persisted for three days during which dihydrostreptomycin was also given. The dihydrostreptomycin was administered with the penicillin for a period of one week. The total duration of penicillin therapy was two weeks. A roentgenogram taken twenty-one days after the acute episode (right center) showed partial clearing of the densities in the lower lung. At this time, pyrazinamide was added to the isoniazid. Four weeks later (right), the lower lung densities had disappeared.

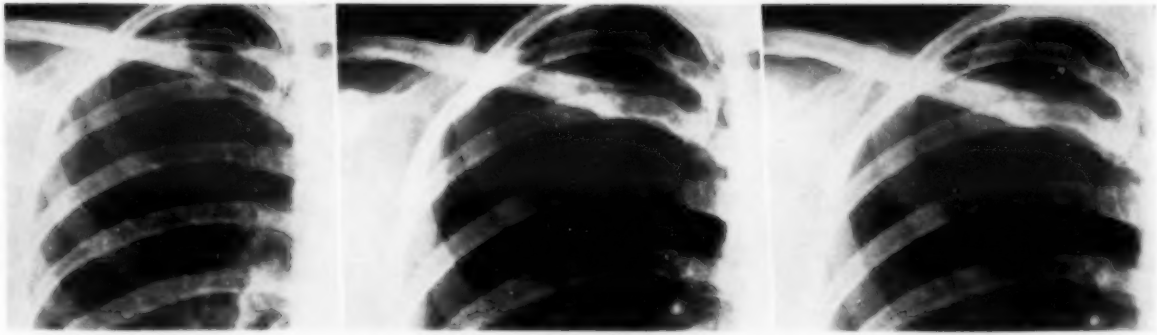


PLATE III

Case 3 (W. G.)

Designated as roentgenographically worse during isoniazid therapy.

A 23-year-old woman whose isoniazid therapy was started on May 5, 1952, because of an apparently recent minimal tuberculous lesion in the left lung (not reproduced). An infiltration beneath right first rib and clavicle was overlooked in the pretreatment roentgenogram (left), attained prominence during therapy, and may be seen in the four-month film (center) and the seven-month film (right). Sputum and gastric washings became and remained negative for *M. tuberculosis* soon after the start of chemotherapy, and the lesion in the left lung (not shown here) receded satisfactorily. The density at the right apex continued essentially unchanged until eighty-one days after the cessation of the year's treatment with isoniazid, when it was noted to have increased in size. An apparently new infiltrate also appeared at the extreme apex at this time. Chemotherapy was reinstituted (streptomycin-PAS) and the density continued to enlarge. It was resected on October 16, 1953, and consisted of a caseous tuberculoma 2 cm. in diameter.

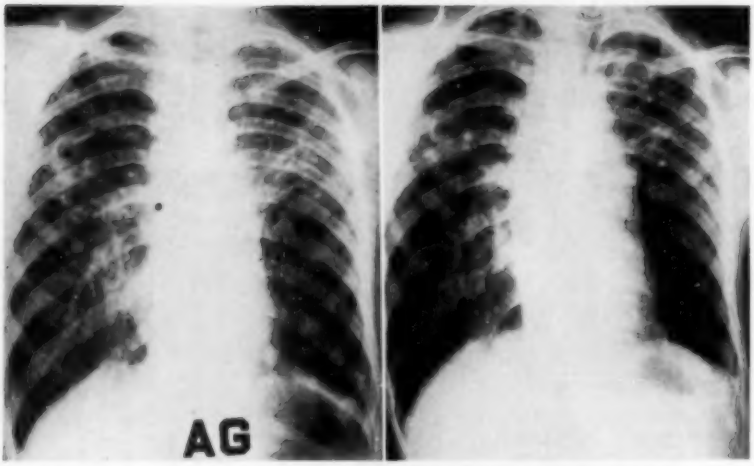


PLATE IV

Case 4 (A. G.)

Designated as roentgenographically worse during isoniazid therapy.

A 17-year-old boy with known pulmonary tuberculosis since July, 1951, which had been treated with streptomycin-PAS. On May 8, 1952, isoniazid therapy was started for bilateral pulmonary tuberculosis with multiple cavities (left). During the succeeding nine months the degree of roentgenographic improvement was designated as slight, i.e., *no significant improvement*. A roentgenogram (right) obtained after nine months of therapy revealed an annular shadow interpreted as representing a new cavity. Accordingly, the designation was changed to roentgenographically *worse* during therapy.

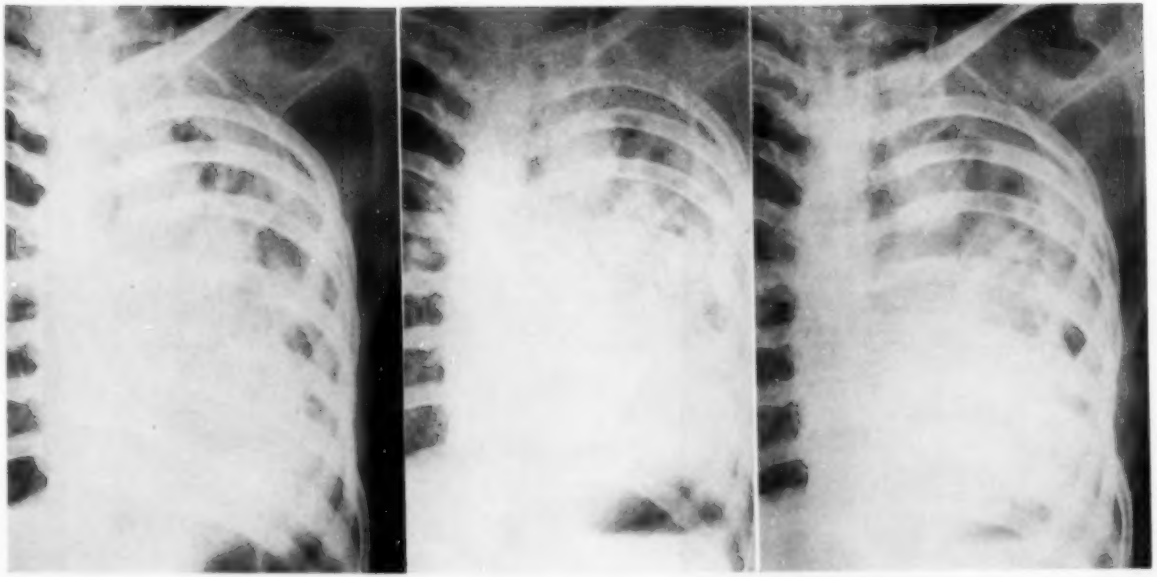
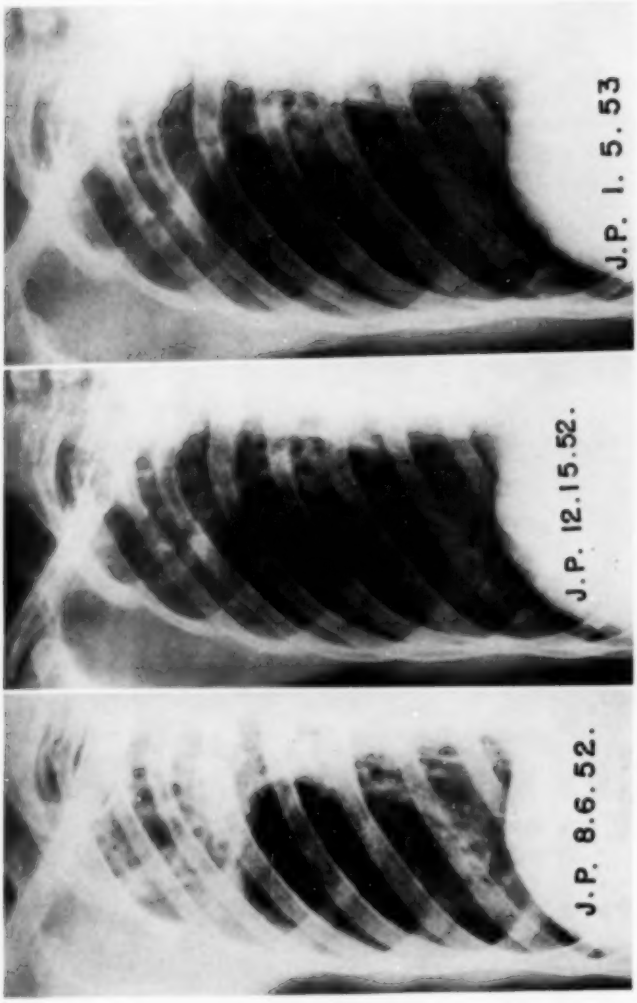


PLATE V

Case 5 (B. B.)

Designated as roentgenographically worse during isoniazid therapy.

A 37-year-old woman known to have had pulmonary tuberculosis for ten years was started on isoniazid therapy on May 29, 1952. The figure on the left is a reproduction of a roentgenogram taken two and one-half weeks after the start of isoniazid. After four and one-half months of isoniazid therapy (center), there was a possible local extension of the disease present in the left lower lung and, at six months (right), excavation was continuing in upper portion of the lung. Subsequent status has remained essentially unchanged.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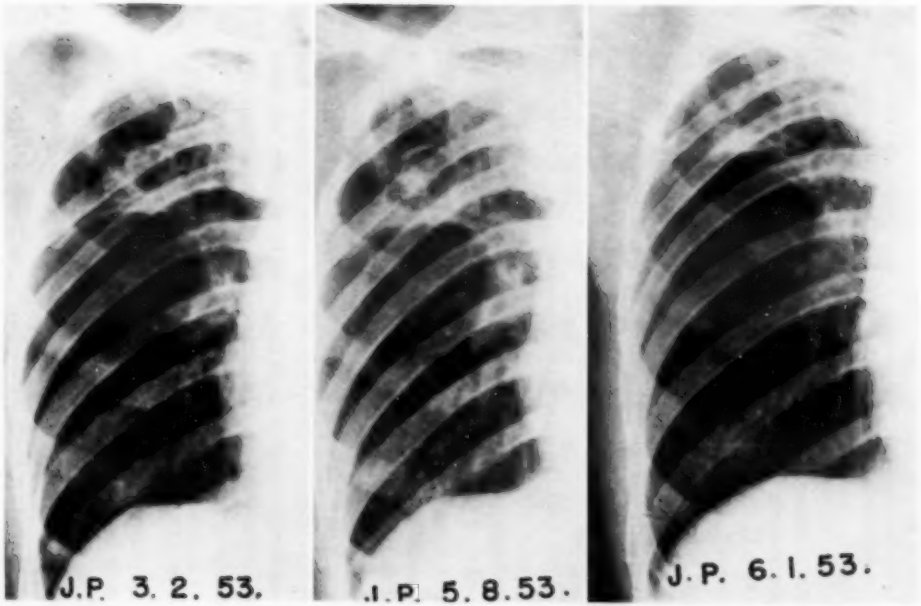

PLATE VI

Case 6 (J. P.)

Designated as roentgenographically worse during isoniazid therapy.

This represents the only instance in the entire series of a change in roentgenographic status from *substantial improvement* to *worse*. The patient was a 28-year-old man whose isoniazid therapy was started on August 7, 1952, for bilateral tuberculosis with the major involvement in the upper right lung (upper left). Tubercle bacilli could be cultured from sputum and gastric washings during the first three months of therapy but not thereafter. The course of the right-sided lesion throughout the entire ten-month period of isoniazid therapy may be seen in the successive films. The over-all collection of densities showed considerable clearing and reduction in area but a cavity appeared in the five-month film (upper right) and showed some increase in size thereafter (figures following). This necessitated changing the three-month roentgenographic evaluation of *substantial improvement*, i.e., *moderate*, to *worse* at six months and subsequently. The patient's chemotherapy was changed to streptomycin-PAS after ten months of isoniazid, but he left the hospital of his own volition shortly thereafter.

METABOLISM OF ISONIAZID IN MAN AS RELATED TO THE OCCURRENCE OF PERIPHERAL NEURITIS[1, 2]

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INTRODUCTION

This report deals with the metabolic fate of isoniazid in a group of tuberculous patients treated with relatively large doses of this drug. The investigation developed from two independent studies; the first on the metabolism of isoniazid in man and monkey, the second on the reactions of the tuberculous patient to doses of the compound somewhat larger than those commonly used. The first of these studies (1) showed unequivocally that N^1-isonicotinyl-N^2-acetylhydrazide is the major metabolic product of isoniazid in the rhesus monkey and suggested that this acetyl derivative is also the major degradation product in man. The second (2, 3) showed that 60 per cent of the patients tolerated isoniazid in daily doses of 20 mg. per kg. for sixteen or more weeks without untoward reaction; the remaining 40 per cent developed peripheral neuritis, in nearly all cases after three to eight weeks of treatment.

Since studies on lower animals (4, 5) had demonstrated that acetyl isoniazid possesses only a small fraction of the toxicity of the parent drug and since work with other therapeutic agents, such as the sulfonamides (6, 7), had shown that humans differ considerably in their capacities to acetylate drugs, the question was raised whether variations in tolerance to isoniazid rest on different capacities of patients to degrade the parent compound. Fortunately, material from the original group of patients was available for study of this question. For reasons pertinent to another investigation (3), twenty-four-hour urine specimens had been collected from a substantial fraction of these patients and aliquots had been stored at 4°C. These specimens were studied for content of isoniazid, acetyl isoniazid, and other metabolic products, with the results described below.

MATERIALS AND METHODS

Patient material: The 17 patients used in this study included 14 with active pulmonary tuberculosis and one each with tuberculous adenitis, meningitis, and renal tuberculosis. The group comprised 11 males and 6 females ranging from thirteen to sixty-one years in age. All infections had been diagnosed re-

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[2] The phases of this study carried out in The Christ Hospital Institute of Medical Research were supported in part by a grant-in-aid (E-509) from the Microbiological Institute, National Institutes of Health, Public Health Service. The parts carried out in the College of Medicine were supported by grants from the Trustees of the Dunham Hospital, Cincinnati, and the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

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cently; none had been subjected previously to specific therapy. In the present study, isoniazid was administered for periods of 19 to 280 days. Sixteen patients received daily doses of 20 mg. per kg.; one received 10 mg. per kg. doses. In all instances these daily doses were divided into three equal portions, administered orally at eight-hour intervals. In addition to isoniazid, each patient received streptomycin, 1 gm. either daily or twice weekly.

Twenty-four-hour urine specimens were collected from 14 of the above patients prior to treatment and from all 17 patients at varying intervals during treatment. Collections were usually made once or twice weekly, but in 3 cases specimens were obtained daily for a period of approximately two weeks. Aliquots of the specimens were stored at 4°C. under toluene and analyzed in groups by the methods described below.

Analytical procedures: The isoniazid and acetyl isoniazid contents of the urine specimens were determined by procedures described heretofore (1, 8, 9). Isonicotinic acid-like materials reacting directly with cyanogen bromide were determined by a slight modification of the method of Rubin, Drekter, Scheiner, and De Ritter (10). As used, this procedure did not measure either unchanged isoniazid or its acetyl derivative. However, in accord with earlier observations by De Ritter, Drekter, Scheiner, and Rubin (11), it was found that pretreatment urine specimens contained nonspecific substances which reacted with cyanogen bromide. In terms of isoniazid equivalent, the twenty-four-hour output of these substances ranged from 7 to 17 mg. The daily excretion of isonicotinic acid-like substances during the treatment period was corrected for the appropriate pretreatment blank, it being assumed, on the basis of the findings of De Ritter and colleagues (11), that the output of nonspecific materials was constant.

RESULTS

The data on the excretion of isoniazid and its derivatives by different patients have been summarized in table 1. Day by day or week by week variations encountered in representative subjects are presented in figure 1. This illustrative material shows that isoniazid undergoes extensive metabolic transformation in man and suggests that metabolism follows a relatively constant pattern in an individual person but that patterns in different persons vary substantially.

Partition of the excretory data, as in table 1, reveals three relatively distinct patterns in the metabolism of isoniazid.

First metabolic pattern: The first, represented by the 6 patients of *Group I*, is characterized by conversion of the drug to two major metabolic products, acetyl isoniazid and "isonicotinic acid-like" derivatives.[4] This metabolic conversion is quite extensive, for, although essentially all of the isoniazid ingested was eliminated in the urine, only 4 to 9 per cent was excreted unchanged. Acetyl isoniazid was the major excretory product in this pattern of metabolism, accounting for 54 to 74 per cent of the parent drug.

Second metabolic pattern: The second metabolic pattern, represented by the 7 subjects in *Group II* (table 1), is characterized by conversion of isoniazid to at least three metabolic products or groups of products. These include the acetyl derivative, "isonicotinic acid-like" substances, and an unknown material(s)

[4] Counter-current distribution studies on the urine of one patient indicate that the substance which reacts directly with cyanogen bromide is actually isonicotinic acid. Studies on other patients are in progress but until the work is complete it seems well to use the designation "isonicotinic acid-like" derivatives.

TABLE 1

URINARY EXCRETION OF ISONIAZID AND ITS METABOLITES BY TUBERCULOUS PATIENTS

GROUP	PATIENT	WEEKS OF TREATMENT	NUMBER OF URINE COLLECTIONS ANALYZED	AVERAGE PER CENT OF DAILY DOSE OF ISONIAZID					OCCURRENCE OF PERIPHERAL NEURITIS
				Recovered in Urine				Fate Unknown	
				Isoniazid (A)	Acetyl Isoniazid (B)	"Isonicotinic Acid" (C)	Total (A + B + C)		
I	Roy	2+	7	3.8	70.6	23.9	98.3	—	—
	McD	24	10	5.2	60.2	36.4	101.8	—	—
	Sl	40	7	5.9	67.2	28.1	101.2	—	—
	Si	2+	6	6.1	64.7	22.5	93.3	6.7	—
	Ro	36	2	9.1	73.9	21.5	104.5	—	—
	Al	27	13	9.2	54.3	37.2	100.7	—	—
II	Wa	7	10	2.9	60.6	10.6	74.1	25.9	+
	Pe	10	5	3.9	42.9	25.5	72.3	27.7	—
	Bl	9	6	7.2	17.5	13.7	38.4	61.6	+
	Jor*	7	6	11.3	27.3	33.7	72.3	27.7	—
	Cov	5	8	15.7	28.2	14.2	58.1	41.9	—
	Bol	3	8	17.1	47.0	15.5	79.6	20.4	—
	Da	3	2	19.0	40.3	25.4	84.7	15.3	+
III	St†‡	6	7	14.0	21.0	?	?	?	—
	Fr‡	5	6	19.6	14.1	?	?	?	+
	Rog‡	8	6	26.5	20.1	?	?	?	+
	Rol‡	17	9	36.8	22.8	?	?	?	+

* This patient received isoniazid in daily doses of 10 mg. per kg.; all others received daily doses of 20 mg. per kg.

† This subject received pyridoxine concurrently with isoniazid.

‡ The urine of these subjects, who had received test doses of tryptophan, could not be analyzed for content of "isonicotinic acid-like" substances due to the presence of interfering tryptophan metabolites.

which has neither an ethylene dichloride-isoamyl alcohol-soluble hydrazine moiety nor a pyridine group which gives as intense a reaction with cyanogen bromide as isonicotinic acid. For obvious reasons the daily excretion of isoniazid and determinable derivatives in this pattern was always significantly less than the intake of the parent drug.

It is noteworthy that, although acetyl isoniazid was the major metabolic product in 5 of the 7 patients, in all but one case, excretion of this derivative was significantly lower than that in the subjects of *Group I*. Conversely, excretion of unchanged isoniazid was generally greater in the subjects of *Group II* than in those of *Group I*. These findings suggest that formation of the third metabolic moiety is accomplished at the expense of acetylation of isoniazid and that metabolism in the direction of the undefined moiety may be more difficult than acetylation (when the latter can be accomplished as in the patients of *Group I*).

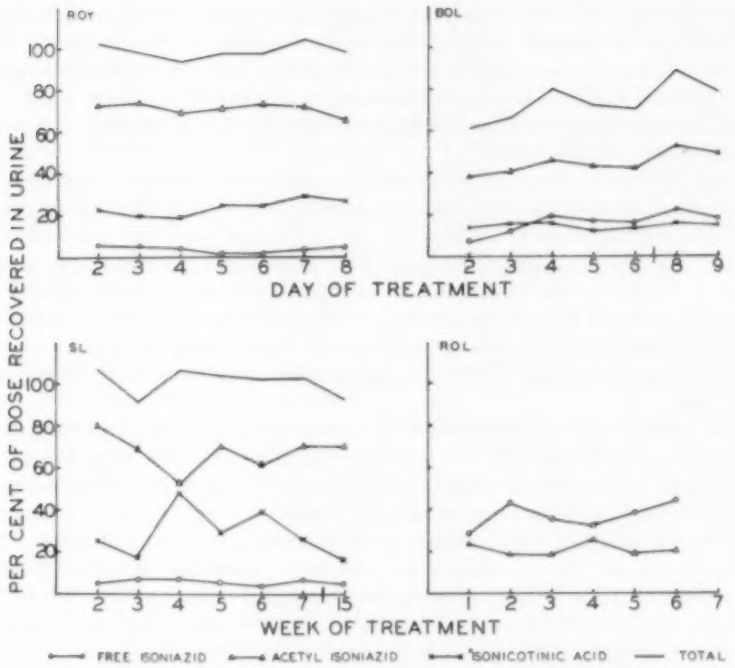

FIG. 1. Daily and weekly variations in the urinary elimination of isoniazid and its metabolites by representative patients.

Third metabolic pattern: The third metabolic pattern, represented by the 4 patients of *Group III* (table 1), can be described with less clarity than the other two due to analytical difficulties[5] which made it impossible to acquire complete data. It appears to be characterized, however, by a slower metabolism of isoniazid and lesser acetylation of this drug than occurred in the other patterns. Thus, in 2 cases, excretion of unchanged isoniazid accounted for more than 25 per cent of the drug intake, while in 3 subjects elimination of the parent drug actually exceeded excretion of the acetyl derivative. This third pattern may be no more than a quantitative variation of the second; however, in the absence of complete analytical data, it has seemed advisable to consider this material separately.

Relation of metabolic patterns to peripheral neuritis: The occurrence of peripheral neuritis among the patients of various metabolic groups is shown in table 1. The contrast between *Groups I* and *III* is especially striking. Thus,

[5] The patients comprising this group had received test doses of tryptophan (3) on the day when the urine was collected. As was demonstrated in control studies, the increased excretion of tryptophan derivatives made it impossible to measure "isonicotonic acid-like" substances. The presence of these derivatives did not affect determination of isoniazid or acetyl isoniazid.

none of the subjects in the first group developed symptoms of peripheral neuritis, whereas 3 of 4 patients in the third group exhibited severe reactions. The absence of neuritic symptoms in the fourth patient (St) may have been related to concurrent administration of pyridoxine, a therapeutic measure which has been remarkably effective in blocking the development of peripheral neuritis during isoniazid therapy (3).

The 7 subjects in *Group II* occupied an intermediary position with respect to incidence of neuritic reactions. Only 3 patients in the group (Wa, Bl, and Da) presented well-developed reactions. Of the other 4 subjects, one (Jor) received only 10 mg. per kg. doses of isoniazid. Two others (Bol and Cov) received the drug for three and five weeks, respectively. As the onset of peripheral neuritis is usually delayed beyond this period (2, 3), and as neuropathies are not commonly produced by 10 mg. doses of isoniazid, the incidence of reactions in the second group might well have been higher with more intensive or protracted treatment.

The findings that peripheral neuritis appeared only among the patients of *Groups II* and *III* and that the disposition of isoniazid in these groups differs significantly from that in *Group I* suggest that this neurologic disturbance is related to the metabolism of isoniazid. It should be noted that the incidence of reactions was highest in the patients who excreted the largest amounts of unchanged isoniazid and the least acetyl derivative. However, peripheral neuritis also occurred in one subject (Wa) who eliminated a substantial fraction of the administered drug as the acetyl derivative and less unchanged isoniazid than any other patient. The common factor in all patients exhibiting neuritic symptoms appeared to be a reduced ability to acetylate isoniazid and, as a consequence, metabolism of the drug to an as yet completely undefined moiety.

DISCUSSION

The data acquired in the present study suggest that at least three processes are concerned with the metabolic disposition of isoniazid in man. The first involves acetylation of the terminal nitrogen of the parent hydrazide; the second, cleavage of the hydrazine group from the parent drug to leave a residue which gives reactions with cyanogen bromide characteristic of isonicotinic acid; and the third, an undefined process (or processes) which results in one or more as yet unidentified metabolites. The first of these processes appears to be the preferred route in the metabolism of isoniazid in man since degradation of the parent compound is most rapid when it predominates and least rapid when it assumes a subsidiary role. The third metabolic process appears least preferable since degradation of isoniazid is slowest when it assumes an important position.

Whereas previous investigations on the fate of isoniazid in man and lower animals have not viewed the total metabolism of the compound in quite the manner described above, the results of earlier studies support the general concepts. Thus, several workers (1, 12, 13) have noted that acetyl isoniazid is one of the major metabolites of isoniazid in man, and experiments in this laboratory (1, 14) indicate that it is essentially the only degradation product in the monkey and is formed with extreme rapidity in both man and monkey. Other studies

(11, 15) have shown that humans and dogs excrete significant quantities of "isonicotinic acid-like" substances. Cuthbertson, Ireland, and Wolff (16) have recently identified isonicotinic acid and isonicotinyl glycine as two of the "isonicotinic acid-like" derivatives in human urine; Ceriotti, De Franceschi, De Carneri, and Zamboni (20) identified isonicotinic acid as an excretory product of isoniazid in the rat. Several investigators (11, 15, 17) attempting balance experiments in man and lower animals have been unable to account for the total dose of isoniazid as either isonicotinic acid-containing or hydrazine-containing excretory products. This finding suggests that either a fraction of the dose has been degraded to an unknown moiety or is stored in the tissues. The latter appears to be no more than a remote possibility.

Comparatively little attention has been given in previous studies to variations in metabolic fate of isoniazid in different individuals. There are indications, however, that such variations do occur both in patients and normal subjects. Elmendorf, Cawthon, Muschenheim, and McDermott (17) found that excretion of hydrazine-containing substances by various tuberculous patients ranged from 48 to 71 per cent of the dose of isoniazid. Working with normal subjects, De Ritter and colleagues (11) found nearly a twofold variation in the excretion of total isonicotinic acid-containing materials, while Demeen, Janssen, Van de Keere, and Zikovic (13) recently reported fourfold variations in the elimination of free isoniazid.

Although there have been numerous reports on peripheral neuropathies induced by isoniazid, no previous attempts have been made to relate the disorders to the metabolic disposition of the drug. The current observations are admittedly preliminary but suggest strongly a relationship between metabolic fate of the compound and occurrence of peripheral neuritis. As indicated previously, it appears that the responsible agent is either one of the presently unidentified metabolic products of isoniazid or some intermediary associated with degradation of parent drug to such a product. The appearance of this agent may actually be due to a defect in acetylating mechanisms resulting from either an absolute deficiency of coenzyme A or an interference by isoniazid with the normal activity of this cofactor. In this connection it is pertinent that a deficiency of pantothenic acid, a component of coenzyme A, induces peripheral neuropathies in the suckling pig (18). It is noteworthy that a deficiency of pyridoxine also promotes peripheral neuropathies (18, 21) and that simultaneous ingestion of substantial quantities of this vitamin prevents neuritis in subjects receiving large doses of isoniazid (3). The mechanism of this effect is obscure; however, studies are currently under way to determine whether pyridoxine or its derivatives alter the metabolic disposition of isoniazid.

Whereas the current study has been concerned primarily with the relation between the metabolic fate of isoniazid and occurrence of peripheral neuropathies, the results have implications for general use of the drug which should be mentioned. Demonstration that isoniazid has diverse metabolic fates in various human subjects suggests that therapeutic responses to the compound would be far from uniform. Since the metabolites of isoniazid eliminated in the urine do not appear to have activity against the tubercle bacillus (19) and since activity

depends on presence of free drug, it should follow, other factors being equal, that therapeutic benefit would be least when the drug is most extensively acetylated and greatest when acetylation and, hence, alternate metabolism are slowest. This thesis is of such significance to the effective therapeutic use of isoniazid that it ought to be tested in a relatively large series of patients. If a relation is established between the metabolic fate and therapeutic effectiveness of isoniazid, modifications of the structure of the drug which would reduce either the rate or extent of acetylation, or attempts to prevent acetylation through use of blocking agents, would seem to be in order. It is recognized, however, that such modification may carry hazards with respect to an increased incidence of peripheral neuropathies.

SUMMARY

The relation of the metabolic fate of isoniazid to the occurrence of peripheral neuritis has been studied in a group of 17 patients treated with relatively large doses of this drug. The results have indicated the existence of three different mechanisms in the degradation of isoniazid. The first and preferred mechanism involves acetylation of the terminal nitrogen of the hydrazine moiety; the second, cleavage of the hydrazine to form isonicotinic acid or a closely related derivative; and the third and most difficult to achieve, degradation to an as yet undefined moiety. The occurrence of peripheral neuropathies was closely associated with an apparent defect in the acetylation of isoniazid and the presence of the third pattern of degradation.

SUMARIO

El Metabolismo de la Isoniacida en el Hombre en Relación con la Ocurrencia de Neuritis Periférica

La relación del comportamiento metabólico de la isoniacida con la ocurrencia de neuritis periférica fué estudiada en un grupo de 17 enfermos tratados con dosis relativamente grandes de esa droga. Los resultados indicaron la existencia de tres mecanismos distintos en la degradación de la isoniacida. El primer mecanismo, que es el preferido, comprende la acetilación del nitrógeno terminal de la mitad hidracínica, el segundo, la disociación de la hidracina para formar ácido isonicotínico o un derivado íntimamente afín, y el tercero, que es el más difícil de realizar, la degradación a una fracción todavía sin definir. La ocurrencia de neuropatías periféricas estaba íntimamente enlazada con un defecto manifiesto en la acetilación de la isoniacida y la presencia del tercer mecanismo.

RESUME

Le métabolisme de l'isoniazide, chez l'homme, en relation avec l'apparition de la névrite périphérique

La relation entre la dégradation métabolique de l'isoniazide et et l'apparition de la névrite périphérique a été étudiée parmi un groupe de 17 malades recevant des doses élevées de ce composé. Les résultats de ces travaux montrent l'existence de trois mécanismes de dégradation de l'isoniazide. Le premier mécanisme, qui est le mécanisme de préférence, comporte l'acétylation de l'azote terminal de la partie hydrazine; le deuxième est le clivage de l'hydrazine qui donne l'acide isonicotinique ou un dérivé très proche; le troisième et le plus difficile à réaliser est la dégradation jusqu'à une fraction encore indéterminée de la molécule. L'apparition des neuropathies périphériques était étroitement associée à une acétylation déficiente de l'isoniazide et à la présence du troisième type de dégradation.

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THE ROLE OF BRONCHOGRAPHY IN PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

Since the advent of resection in the treatment of pulmonary tuberculosis, it has become increasingly essential to make a preoperative anatomic diagnosis of the extent of the disease. Although bronchographic assessment of adult tuberculous patients was reported sporadically from 1927 onwards, the procedure was generally considered inadvisable in view of the danger of causing spread of disease by the introduction of a mechanical irritant or through the deleterious effect of iodine-containing substances on the tuberculous infection.

Neuhoff (1) predicted in 1934 that "the increased application of mechanical methods (of treatment) . . . .would place the bronchogram on a par with the Roentgen film." Barnwell and his associates, in 1937, mentioned its use in the demonstration of bronchial stenosis, but it was not until 1944 and 1945 that Dormer and his associates (2, 3) published a large series showing the incidence of bronchial dilatation in various types of tuberculous lesion. Dormer and his associates emphasized the frequency of bronchographic change even in cases with "minimal" infiltration and also the "bronchial" nature of many small cavities seen on the plain film.

Rabinowitch and Harper (4) reported 100 bronchograms on patients who were being considered for resection, or who continued to discharge tubercle bacilli following collapse therapy. Eighty-three of these bronchograms were abnormal and, of 54 patients in whom bronchoscopy showed no disease, 41 had abnormal bronchograms. Jones, Peck, and Willis (5) stated that, in some cases in which bronchiectasis was shown in the bronchogram, histologic examination revealed "spaces lined with necrotic debris—no remnants of the bronchial wall could be found." These writers relate the incidence of bronchiectasis to the duration of the disease and stress the importance of intrinsic factors in the production of bronchial dilatation and the absence in the majority of cases of a proximal obstructive element, in contradistinction to their findings in bronchiectasis associated with the primary infection. Buckles and his associates (6), however, concluded that in the majority of their cases an obstructive lesion was or had been present, and called these cases "tuberculous bronchitis with bronchiectasis," as opposed to a minority in which "true tuberculous bronchiectasis" with caseation involving the whole bronchial wall was found.

Marchese and his associates (7) reviewed the reported incidence of undesirable reactions following bronchography. The majority of complications were due to spread of the disease, although there were a few cases of iodism, none of them fatal, and occasional instances of lipoid granuloma formation. These writers concluded that postbronchographic spread occurred only in the presence of a sputum positive for tubercle bacilli at the time of bronchography. All of the patients with reactions or complications had undergone bronchography in the days when antimicrobial therapy had not been available to assist in the control of the lesion prior to the procedure.

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The present investigation was conducted for the purpose of defining the value of bronchography in pulmonary tuberculosis, to determine its limitations and dangers, and to reach a conclusion regarding its usefulness, with special reference to its application in the surgical treatment.

The material consisted of 100 adults with pulmonary tuberculosis in whom satisfactory bronchograms were obtained. A high standard of technical excellence was insisted upon and, for this reason, 13 of 113 cases (11.5 per cent) were discarded as being inadequate. The review was largely retrospective, but in the majority of cases it was possible to assess the value of the bronchograms before seeing the operative and pathologic reports.

Method

Dionosil® was the contrast medium used throughout the present investigations except for 3 occasions when sulfadiazine suspended in iodized oil was substituted. Dionosil® "aqueous" and Dionosil® "oily" are suspensions of propyliodone in aqueous and oily vehicles, respectively. Propyliodone is the N-propyl ester of 3:5-diiodo-4-pyridone-N-acetic acid. The properties of this new contrast medium are now well recognized. The fact that the substance is absorbed and eliminated completely from the lung fields in twenty-four to forty-eight hours has established its use in pulmonary tuberculosis. When first introduced, Dionosil® was used only in the aqueous form. The alternative oily solution was used later as it was found less likely to provoke coughing.

All of the patients in the series were being treated as inpatients at the time when bronchography was carried out. The procedure was employed only if the patient was afebrile and the disease was considered to be in a quiet phase. Premedication with Pantopon® and scopolamine was used as a routine. One hour after the premedication the patient was given a decicain lozenge (0.065 gm.). The larynx, cords, and trachea were thoroughly anesthetized with Xylocaine® 4 per cent solution. A Jaques catheter, size 6-8, was passed through a previously anesthetized nostril and into the trachea, using the "blind" method of intubation. Only by intubating the patient is it possible to have complete control over the filling of segmental bronchi and bronchioles, which the writers consider to be absolutely essential before attempting to interpret bronchial changes.

A total of 14 to 18 cc. of Dionosil® was found to be the average amount necessary for the complete filling of either bronchial tree. One of the usual methods of posturing was adopted, special attention being paid to the filling of the upper lobes by lowering the head of the table to ensure adequate filling of the smaller apical bronchi and bronchioles.

On return to the ward, each patient was "tipped" until as much as possible of the contrast medium had been expectorated. During the initial stages of the study a routine chest roentgenogram was obtained twenty-four hours later, but this practice was found to be unnecessary and was discontinued.

OBSERVATIONS

Complications

Of the 100 patients, 72 suffered no ill effects whatsoever. The complications which arose in the remainder were as follows:

Twenty-two patients had a transient fever of less than 100°F. and 3 had a fever greater than 100°F. In neither of these groups was there any constitutional upset. In 2 patients with widespread bilateral bronchial changes, the fever was accompanied by bronchitis, bronchospasm, and influenza-like symptoms such as headache and backache. A third patient with a fever of 102°F. was found on roentgenographic examina-

tion twenty-four hours later to have a segmental collapse in the lower lobe on the side on which the bronchogram had been performed. The atelectasis was transient and cleared up within three days. A spread of tuberculous disease occurred in only one patient. In this instance, sulfadiazine suspended in iodized oil had been used. Incidentally, this was the only occasion when a large tuberculous cavity was outlined with contrast material. The disease was bilateral, widespread, and active.

In general, the patients in whom these complications occurred were those in whom the tuberculous disease was widespread. Therefore, when the disease is of this extent, bronchography should be used only if essential. Two patients with sputum previously negative for *M. tuberculosis* on culture were found to have acid-fast bacilli in the sputum on microscopy, a few days following the bronchogram.

Interpretation of Bronchograms

The bronchial changes in diseased segments fall naturally, but not inevitably, into a progressive pattern which can be loosely correlated with the duration and severity of the disease. This is not to say that the pathology can be predicted from the bronchograms alone with any useful degree of accuracy. Furthermore, the bronchographic findings may be produced by two types of pathology: one in which the bronchial changes are centripetal and associated with peripheral pneumonic lesions, and one in which the bronchial changes are secondary to previous proximal endobronchial disease or bronchial compression. It must be emphasized, however, that the writers do not propose to make pathologic deductions from the bronchographic findings.

It was decided to classify the bronchographic appearances on an arbitrary division into three grades, as set forth in table 1.

TABLE 1

CLASSIFICATION OF BRONCHOGRAPHIC APPEARANCES IN 100 CASES OF PULMONARY TUBERCULOSIS

GRADE	TYPE OF CHANGE	NUMBER OF CASES
1	(A) Beading of peripheral bronchi (B) Slight distortion of bronchial passage, progressing to crowding (C) Occlusion of peripheral bronchi	23
2	(A) Irregular dilatation of peripheral and intermediate bronchi (B) Moderate to severe distortion and crowding (C) Occlusion of bronchi at level of second and third divisions	43
3	(A) Gross saccular bronchiectasis (B) Gross crowding (C) Occlusion of bronchus up to segmental orifice	34
Total		100

TABLE 2

INFORMATION DERIVED FROM THE BRONCHOGRAMS IN 100 CASES OF PULMONARY TUBERCULOSIS

GROUP	NUMBER OF CASES
(*A*) New information of value in assessment	77
(*B*) Confirmatory evidence only	19
(*C*) Misleading	4
Total	100

The distinction between bronchial occlusion and technical failure to fill the bronchi is not always easy, especially when minimal changes in the upper lobe are present. When adjacent segments were well filled and the bronchus immediately proximal to an occlusion showed other evidence of abnormality, occlusion in the affected area was assumed.

The information derived from the bronchogram was evaluated as shown in table 2.

The 4 cases in which the information obtained was misleading (*Group C*) are discussed below. The cases in which only confirmatory evidence was obtained include those cases in which a confident anatomic assessment of the disease had been made by standard roentgenography and tomography (*Group B*).

The majority were in *Group A*, i.e., new information of value. The information derived from the bronchograms in this group can be classified as follows:

Anatomic localization (27 cases): The bronchogram was successful in localizing disease on a segmental basis when other roentgenographic techniques had failed to do so. This was especially so when the bronchial tree was distorted by previous collapse therapy or its complications and by gross contraction and fibrosis due to disease.

Demonstration of unsuspected areas of bronchial damage (29 cases): When disease has previously been present, its healing may be accompanied by clearing of roentgenographic shadows. The finding of persistent bronchial damage on the bronchogram is an index of previous infection. When these changes are localized to the apex of the lower lobe, their discovery is of considerable value, as in this segment the original disease may have been overlooked.

Demonstration of healthy segments (21 cases): The well-known tendency for anteriorly placed segments to escape involvement by tuberculosis was borne out by the large number of cases in which bronchial damage was found to be absent in these areas. In upper lobe disease the finding of a normal anterior segmental bronchus has made the surgeon feel justified in conserving a large volume of healthy lung.

Patients subjected to surgery: It was possible to make a critical analysis of the 56 cases which came to thoracotomy (table 3).

Of the 44 cases in *Group A* (new information of value), 13 showed disease previously unsuspected; 13, an uninvolved segment; and in 18 the localization of disease was precisely defined.

In 37 cases a definitive operation was planned on the bronchographic findings. In 33 of these the findings were exactly as predicted. In 4 cases, segmental resec-

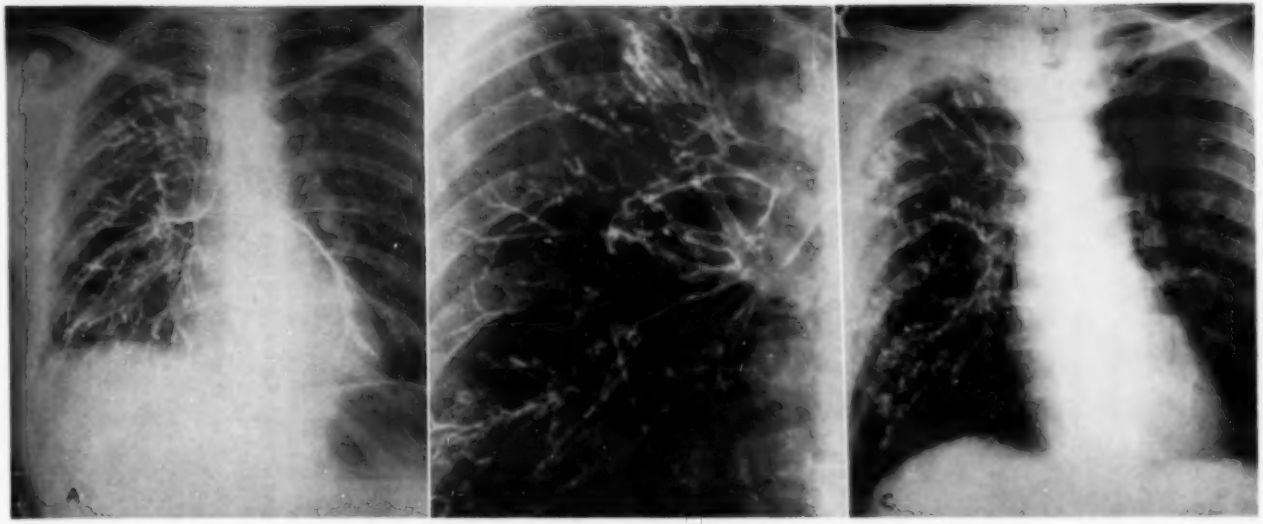

FIG. 1

A. Minimal changes involving apical and posterior segmental bronchi of the right upper lobe.
B. Minimal crowding of branches of posterior segment of the right upper lobe.
C. Occlusion of peripheral bronchi apical and posterior segments of the right upper lobe.

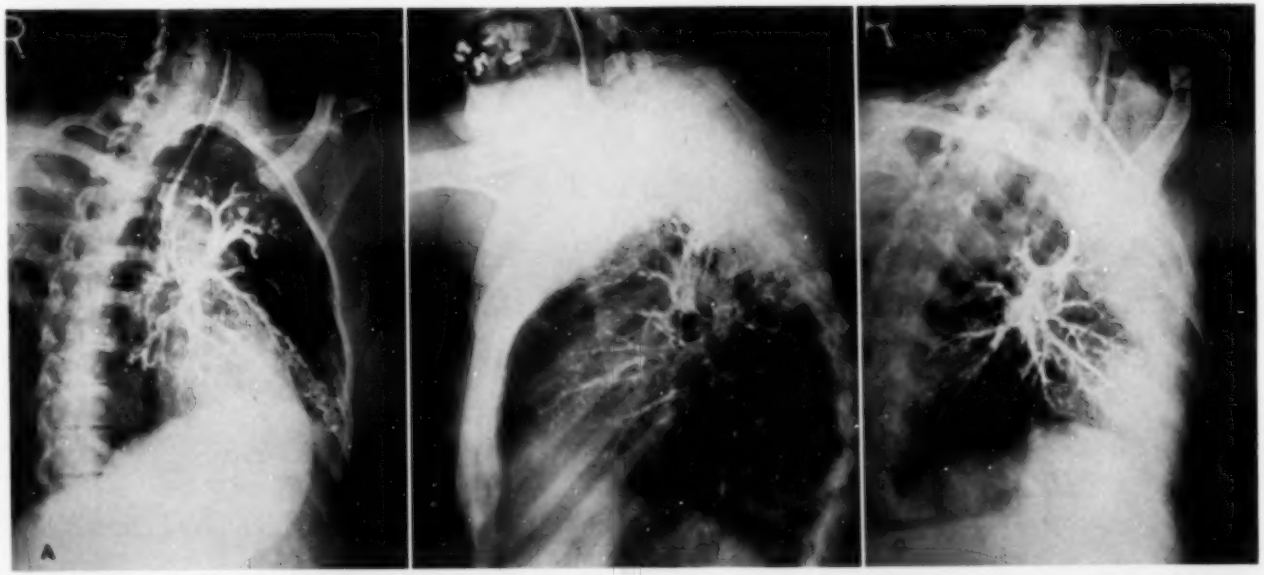

FIG. 2

A. Irregular dilatation of peripheral and intermediate bronchi of the left upper lobe; healthy lingula and apex of lower lobe.
B. Crowding of all segments of the left upper lobe; the lingula occupies the position of the displaced anterior segment.
C. Occlusion of segmental bronchi of the left upper lobe at level of second division.

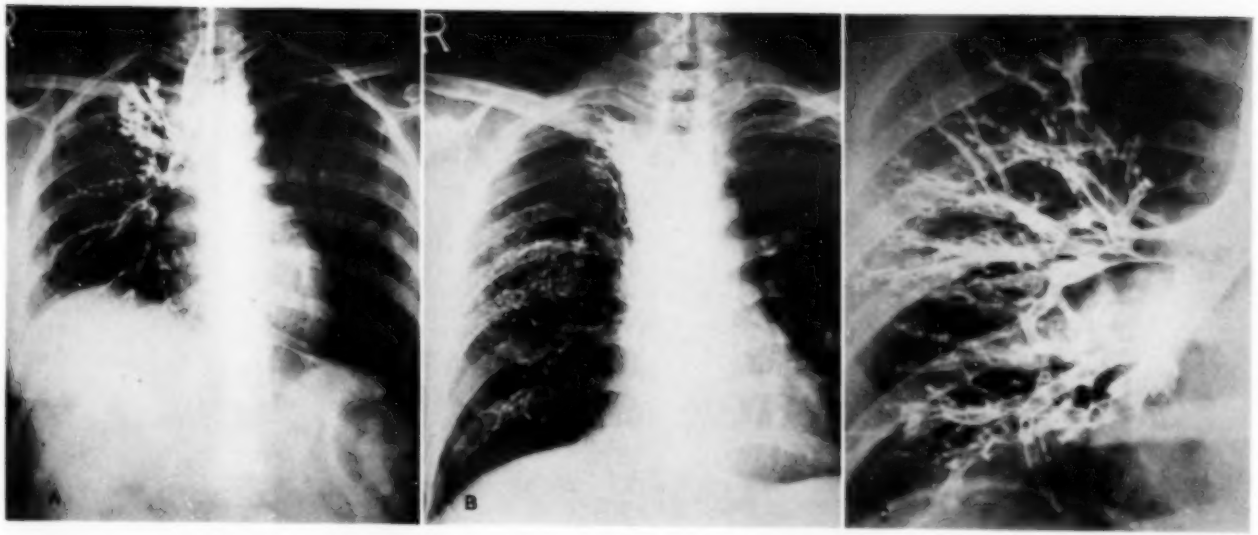

FIG. 3

A. Gross saccular bronchiectasis involving the right upper lobe and the apical segment of the lower lobe. Note the anomalous origin of the apical segmental bronchus of the upper lobe.

B. Gross crowding of all segments of the right upper lobe. The collapse of the lobe was not clearly demonstrated on plain roentgenography.

C. Occlusion of apical, anterior basal, and posterior basal segments of the right lower lobe, at level of segmental orifices.

FIG. 4 The bronchogram of M. A., showing Grade 2 changes in the posterior and anterior segments of the right upper lobe. At thoracotomy, the whole right upper lobe at first appeared involved but, on removal of the posterior and anterior segments, a palpably normal apical segment remained.

tion of the lobe was predicted, but lobectomy was performed on the finding of subpleural nodules in all segments.

There were 4 cases in which the information was considered misleading. In one, in which a left lower lobectomy was done, the bronchogram failed to demonstrate disease in the left upper lobe which was found at operation. In another instance, the lingula was mistakenly identified as the anterior segment of the upper lobe. At operation, the lingula was found to be diseased, whereas the anterior segment of the upper lobe was normal. In the third, the middle lobe was thought on the bronchogram to be diseased, but at operation appeared to be healthy on palpation. In the last case, disease was found in all segments in the upper lobe at operation, whereas the bronchogram had been interpreted as showing disease in the apical and posterior segments only.

TABLE 3

ANALYSIS OF 56 CASES WHICH CAME TO THORACOTOMY

GROUP	NUMBER OF CASES
(*A*) New information of value in assessment	44
(*B*) Confirmatory evidence	8
(*C*) Misleading	4
Total	56

TABLE 4

TYPE OF OPERATION PERFORMED IN 75 CASES OF PULMONARY TUBERCULOSIS

TYPE OF OPERATION	NUMBER OF CASES
Thoracoplasty	22
Resection	
Segment(s)	21
Lobe(s)	29
Lung	3
Total	75

The type of operation performed in 75 cases submitted to surgery is shown in table 4.

When thoracoplasty was performed, the decision was often influenced by the bronchographic demonstration of such widespread abnormalities that it was considered that insufficient healthy tissue would be left were resection carried out.

DISCUSSION

The information to be obtained from bronchography is of two types: anatomic and pathologic. In this paper, no attempt has been made to correlate the bronchographic appearances with pathologic findings, as a precise correlation can only be achieved by an extensive study of operation specimens. Attention, therefore, has been directed to the anatomic aspect, and it is submitted that here bronchography offers an advance in the roentgenographic assessment of the patient in whom surgical treatment, especially resection, is being considered. It should be emphasized that abnormalities of the bronchial tree were frequently found in association with roentgenographic disease hitherto considered to affect only the parenchyma. These abnormalities were present at any level in the bronchus, from the periphery to the hilum. It cannot be claimed that these changes are peculiar to tuberculosis; similar changes may occur in other forms of chronic bronchial disease, but the frequency with which they were localized to areas of the lung known to be the site of tuberculous infection indicate that they are probably due to tuberculosis per se. Bronchial changes were present in the majority of the cases studied. In only 4 cases was there a failure to demon-

strate bronchial abnormalities in segments discovered at operation to be the seat of tuberculous disease. As the series was preselected in so far as bronchograms were done in only those cases in which surgery was contemplated, it is not possible to form any opinion of the over-all frequency of bronchial involvement as demonstrated by the bronchogram in pulmonary tuberculosis.

The value of the bronchogram lies chiefly in the definition of the segmental localization of disease (*see* figure 4). It may be argued that tomography in various planes offers an adequate means of defining segmental anatomy. It is suggested that, when the bronchial tree is distorted, for instance, under a thickened pleura or pleural effusion, or when paramediastinal collapse is suspected, the most accurate means of establishing the exact anatomy is by bronchography. Again, when isolated disease lies close to a fissure, the bronchogram remains the best means of demonstrating its precise position. When upper lobe disease and apex of lower lobe disease coexist, the lower lobe disease may not readily be detected on plain roentgenography and tomography alone. Resection performed in such cases may involve a more extensive operation than is anticipated, and re-expansion of the remaining portion of the lung to fill the pleural space is frequently a problem.

Conservation of healthy lung tissue must be a major consideration in the surgical treatment of the tuberculous patient; in many of the patients who came to thoracotomy, it could be confidently predicted before operation that healthy segments existed which could safely be left *in situ*. Finally, when widespread bronchial involvement is encountered, it may explain an otherwise puzzling degree of respiratory insufficiency.

There are few disadvantages to this method of investigation. The spread of tuberculous disease has hitherto been considered to be a major hazard but has occurred in only one of the patients as mentioned above. In the early stages, bronchograms were performed chiefly to show the state of the bronchi and, by inference, the lung. It was expected that a pulmonary segment with a normal bronchial tree would prove eventually to be free of tuberculous disease. This, although a useful working hypothesis, was not found to be strictly true, as a normal bronchial tree is compatible with small nodules in the lung tissue. It might be reasonable, however, to postulate that lung units with normal bronchographic outlines do not contain the type of disease that warrants surgical intervention.

SUMMARY

The application and the value of the bronchogram in pulmonary tuberculosis are reviewed in 100 patients under assessment for surgical treatment of their disease.

The frequency of bronchial abnormality is emphasized, and the bronchographic appearances are classified.

Bronchographic investigation provides a method of segmental localization of disease unsurpassed by other available roentgenographic procedures.

The use of bronchography is recommended in all cases of pulmonary tuber-

culosis when segmental resection is contemplated and precise anatomic definition is lacking.

SUMARIO

El Papel de la Broncografía en la Tuberculosis Pulmonar

Discútese la aplicación de la broncografía en la tuberculosis pulmonar repasándose su valor en 100 enfermos en vías de estudio para tratamiento quirúrgico de la dolencia.

Recálcase la frecuencia de la anomalía bronquial y se clasifican los aspectos broncográficos. La investigación broncográfica facilita un método para la localización segmental de la enfermedad, cuyo valor no superan los otros procedimientos radiográficos disponibles.

Recomiéndase el uso del mismo en todos los casos en que se considera la resección segmental y falta la definición anatómica precisa.

RESUME

Le rôle de la bromchographie en tuberculose pulmonaire

Les applications du bronchogramme en tuberculose pulmonaire sont étudiées. Sa valeur est considérée chez 100 malades soumis à l'évaluation d'un traitement chirurgical dirigé contre cette affection.

La fréquence des anomalies bronchiques est soulignée et les images bronchographiques sont classifiées. Cette étude procure une méthode de localisation segmentaire de l'affection supérieure à tout autre procédé radiographique en usage.

Son emploi est recommandé dans tous les cas où la résection est envisagée et où une détermination anatomique precise fait défaut.

Acknowledgments

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SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS[1, 2]

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INTRODUCTION

Evaluation of segmental surgical excision in pulmonary tuberculosis is now a current problem. Therefore, the writers have studied 70 consecutive patients who underwent removal of 130 portions of tuberculous lung of segmental, multiple segmental, or smaller size. The purpose of this paper is to report the pertinent roentgenographic, pathologic, and bacteriologic findings together with the duration and kind of disease, type, extent, results, and complications of pulmonary excision. The data are organized, not only to provide additional evidence for evaluating the place of sublobar surgical excision of the tuberculous lung, but also to shed more light on the problem of resection of residual necrotic lesions (1, 2).

MATERIALS AND METHODS

The data concerning age and sex show no unusual distribution, and 3 patients who were more than sixty years of age withstood the operation well (table 1).

Duration of disease: The duration of disease since first diagnosis, before excisional surgery, is shown in table 2. These data prove that these cases were chronic, with approximately 89 per cent (all except 8 early primary cases) of the patients having had a duration of disease of more than one year. Added to this table is the classification of Overholt, Woods, and Wilson (3) for separation of the segmental operation into primary, secondary, or early primary. Secondary operations were preceded by collapse or resection procedure other than pneumoperitoneum in 21 cases, 30 per cent of the 70 patients. Forty-one (59 per cent) had a primary excision; of these, 17 (41 per cent) had a duration of disease of four or more years. In the secondary group of 21 cases (30 per cent of the entire group), 17 patients (81 per cent) had a duration of disease of more than four years. Thus, the secondary group of patients had a much longer duration of disease than the primary group.

Antimicrobial therapy: All patients had some antimicrobial therapy. If not given months (figures 1 and 2) or years before segmental resection, it was always given at the time of surgery and was continued for varying periods thereafter. Thirteen patients (19 per cent) who had less than one week of antimicrobial therapy before surgery were recorded as zero duration in the column "0–4" in the data of table 3. All 13 of these patients had sputum negative for tubercle bacilli preoperatively and postoperatively. More than five months of antimicrobial therapy preceded surgery in 26 (37 per cent) of the entire 70 cases. Forty-four patients (63 per cent) had antimicrobial therapy zero to four months. The usual form of therapy since February, 1952, has been "triple treatment" consisting of streptomycin, usually 2 gm. per week; para-aminosalicylic acid (PAS), 12 gm. per day; and isoniazid, 300 mg. per day. Preceding February, 1952, streptomycin, 1 gm. five times weekly, and PAS, 16 gm. per day, was the regimen employed.

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[1] From the Thoracic Surgical, Laboratory, and Medical Chest Services, City of Hope Medical Center, Duarte, California.

[2] Presented before the Medical Session, as part of the session on *Surgery in the Treatment of Tuberculosis*, at the annual meeting of the National Tuberculosis Association, on May 19, 1953.

FIG. 1. Surgical pathology specimen showing a blocked cavity filled with soft caseous material highly positive for tubercle bacilli on smear and culture. The patient was a 34-year-old white female who had a secondary right upper lobe apical segmentectomy. Preoperative sputum and gastric washings were negative for tubercle bacilli for ten years. Preoperative antimicrobial therapy was given for nine and one-half months.

FIG. 2. Surgical specimen showing the right upper lobe apical segment with partially stenosed bronchus draining the cavity. The patient was a 23-year-old Chinese female with sputum consistently positive for tubercle bacilli preoperatively in spite of seven and one-half months of antimicrobial treatment during a nine-month period. Duration of disease was three years. Cavity smear and culture were highly positive for *M. tuberculosis*.

TABLE 1

SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS IN 70 PATIENTS
Age and Sex at Time of Surgery

AGE	MALE	FEMALE	TOTAL	REMARKS
years				
15–20	1	2	3	Youngest, 18 years. Oldest, 66 years. Largest group, 25 to 40 years
21–25	1	3	4	
26–30	7	7	14	
31–35	5	6	11	
36–40	3	12	15	
41–45	3	4	7	
46–50	5	1	6	
51–55	3	2	5	
56–60	2	0	2	
61–65	1	1	2	
66–	1	0	1	
Total	32	38	70	

TABLE 2

SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS IN 70 PATIENTS
Duration of Disease Before Segmentectomy

	TIME IN YEARS						TOTAL CASES
	1–4	4–8	8–12	12–16	16–20	20–24	
Secondary	4	6	4	2	2	3	21
Primary	24	9	6	1	1	0	41
0–12 months early primary	8						8
Total							70

TABLE 3

SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS IN 70 PATIENTS
Duration of Antimicrobial Treatment Before Surgery

	MONTHS					TOTAL CASES
	0–4	5–8	9–12	13–16	17–24	
Secondary	15	5	1	0	0	21
Primary	22	11	7	0	1	41
Early primary	7	1				8
Total						70

Operative technique: Several techniques of operations were performed, which could be divided into: lobectomy, plus one or more segments; lobectomy, plus one or more excisions or enucleations less than segments; one segment only; two segments only; one or more segments, plus excisions less than segments; three segments; wedges and excisions or enuclea-

TABLE 4

SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS IN 70 PATIENTS
Operative Techniques of Excision

TYPE OF EXCISIONAL SURGERY	NUMBER OF CASES	REMARKS
Lobectomy plus one or more segments	8	11 segmentectomies 1 wedge segmentectomy with clamps
Lobectomy plus one or more excisions or enucleations less than segments	4	2 excisions with wedge and clamps 15 enucleations without clamps
One segment only	19	7 cases Apical*—posterior left upper lobe
Two segments only	15	
One or more segments plus excisions less than segments	6	5 wedges with clamps 5 enucleations without clamps
Three segments	4	2 upper lobes in lungs without fissures
Wedges and excisions or enucleations less than segments	14	25 enucleations without clamps 10 wedges with clamps

* Jackson-Huber classification.

tions less than segments (table 4). Two tubes were routinely used for pleural drainage with suction.

One hundred and thirty portions of diseased lung were excised from the 70 patients. Approximately forty of these were encapsulated nodules 1 to 2 cm. in diameter; the other ninety were of segmental or lobar magnitudes. Decision to perform enucleations or excisions of nodular disease was made by the surgeon at the time of surgery.

The apical-posterior segment of the left upper lobe was considered as one segment only, according to the Jackson-Huber (4) classification. In conserving lung by these sublobar excisions, due regard was given for the expansion of remaining segments in the homologous lobe, but occasionally a small, fleshy, fibrotic subsegment of atelectatic lung was left in the apical segment of the lower lobe without apparent sequelae.

The technique described by Churchill in 1939 (5), who orginated segmental resection, was satisfactory in most instances. The modifications described by Overholt and Langer (6) and Chamberlain and Klopstock (7) were also used. Such subsegments were usually resected without oversewing the visceral pleura and with a minimum use of clamps.

RESULTS

Complications: There were 121 different complications (table 5). As in every segmental resection airtight closure of the visceral pleura was not attempted, air always leaked into the pleural cavity from fractured, sutured, or incised lung. If there was no detectable air leak found at the time, the pleural tubes were removed, usually by the fourth day, and the air leak was not recorded as a com-

TABLE 5

COMPLICATIONS AND END RESULTS OF SURGERY IN A SERIES OF 70 SEGMENTAL OPERATIONS

COMPLICATIONS	NUMBER OF CASES	END RESULT OF COMPLICATIONS	EFFECT OF COMPLICATIONS ON CASE FOLLOW-UP
			Poor
Air leaks			
Transient	9	All closed in five to ten days without further treatment	2
Persistent	3	2 Eloesser flap (B.P.F.); 1 secondary closure and 5-rib thoracoplasty (successful)	
Pleural reaction and thickening	39	All cleared within three months	
Pleural fluid	24	All cleared within two months	
Atelectasis	5	Temporary	
Air space (nonexpanding lung)	12	10 re-expanded within one and one-half months; 2 responded to closed thoracotomy and re-expanded	
Postoperative hemorrhage	4	3 responded to whole blood transfusions; 1 died in six hours postoperatively	1
Subcutaneous emphysema	2	Both cleared in three days	
Hematoma of lung at operative site	4	All cleared roentgenographically within three months	
Reactivation	2	Both had bilateral disease presurgically	
Thyroid crisis	1	Responded well to radioactive iodine therapy	
Incisional pain	1	Patient was an emotional problem presurgically	
Excessive shock	1	Patient (also had postoperative hemorrhage*) died six hours postoperatively	
Leak or contamination at surgery (ruptured cavity or necrotic foci, et cetera)	7	6 no effect; 1 died five and one-half months postoperatively (tuberculous meningitis)	1
Patients with sputum positive for tubercle bacilli post surgery	6	All have bilateral disease	
Cor pulmonale right heart failure	1	Died forty-eight hours postoperatively	1
Total	121		5

* Counted with postoperative hemorrhage.

plication. If the air leak persisted for more than five days but did not progress to another pleural complication, it was classified as "transient." If the air leak appeared to be associated with opening of the segmental bronchial stump, or developed into more serious pleural complication, it was classified as "persistent."

Of the 3 patients with persistent air leaks, 2 had bronchopleural fistula with empyema and were successfully managed by Eloesser flap drainage. Success in the third patient was attained by thoracotomy, suture of the bronchopleural fistula, and simultaneous five-rib thoracoplasty.

Pleural reaction or thickening occurred in 39 patients (56 per cent). Twenty-four patients, some of whom are recorded as having pleural thickening, also had pleural fluid. Within two to three months, these minor pleural complications usually disappeared and had no serious effects on the clinical course. Roentgenographically, some permanent pleural changes could usually be detected.

Serious complications included postoperative hemorrhage, clotted hemothorax, empyema, excessive shock, right heart failure, and meningitis. Postoperative bleeding and excessive shock were combated successfully in 3 patients by whole blood transfusions, vasoconstricting drugs, and whole adrenal extract, but one additional patient died six hours postoperatively. An additional death occurred in a patient who had a twelve-year duration of bilateral disease and cor pulmonale, underwent lobectomy with segmentectomy, developed right heart failure, and died forty-eight hours postoperatively.

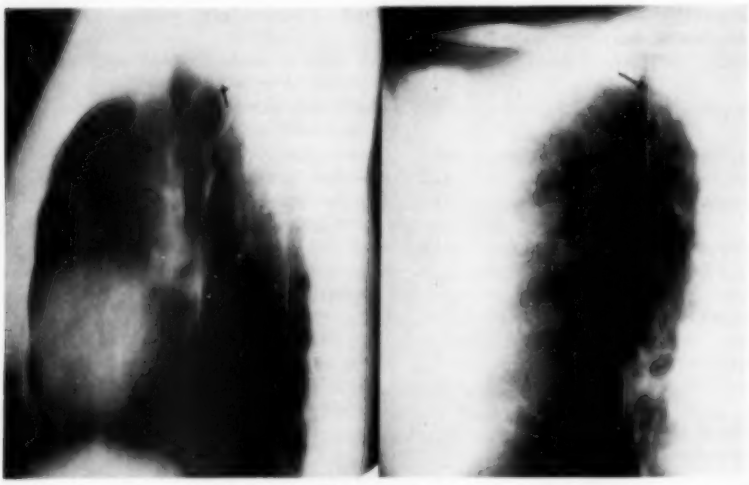

FIG. 3. (Left) Preoperative lateral planigram of the chest showing localized necrotic caseous lesion. Same case as figure 2.

FIG. 4. (Right) Preoperative postero-anterior planigram of the chest at level 7 showing indistinctly localized necrotic caseous lesion in the apical segment of the right upper lobe. Same case as figures 2 and 3. Note the failure of even these more sensitive types of roentgenographic examinations to demonstrate the disease seen in figure 2, which shows an air-containing cavity.

TABLE 6

SEGMENTAL RESECTION FOR PULMONARY TUBERCULOSIS
Correlation of Roentgenographic and Pathologic Findings of Surgical Specimens in 39 Cavitary Cases

	ROENTGENOGRAPHIC DIAGNOSIS	PATHOLOGIC FINDINGS IN SURGICAL SPECIMENS	CORRELATION BETWEEN ROENTGENOGRAMS AND PATHOLOGY
	Cases	*Cases*	*Cases*
Poorly localized and/or nonencapsulated caseous lesions	14	16	9
Encapsulated caseous foci	54	59	54
Cavities	33	39	26
Parenchymal fibrosis	35	54	32
Atelectasis	1	34	1
Bronchiectasis	2	15	1

Of 7 cases of contamination of pleural cavity at surgery, in 6 there were no untoward effects, but one patient died of tuberculous meningitis five and one-half months after operation, although his sputum was negative for tubercle bacilli since the segmental resection.

Correlation of roentgenographic and pathologic findings: An analysis of the correlation between the roentgenographic diagnosis (figures 3 and 4) and the pathologic findings in the surgical specimens (figures 1 and 2) was made utilizing routine and planigraphic studies. This correlation included six morphologic categories which could be diagnosed both roentgenographically and pathologically in the surgical specimen (table 6). The usual excess of extent of disease found over extent of disease predicted by roentgenography was observed. Of 16 poorly localized and/or nonencapsulated caseous lesions, only 9 (56 per cent) were predicted by roentgenography. Of the 14 such roentgenographic diagnoses, only 9 (64 per cent) were correct.

Of the 59 cases in which encapsulated caseous foci were demonstrated in the surgical specimens, 54 (92 per cent) were diagnosed by roentgenography. In the surgical specimens, 4 so-called encapsulated caseous foci were demonstrated to be former cavities which had become blocked by fibrous obliteration of the bronchocavitary junction.

Of the 39 cavitary cases found in the surgical specimens, 26 (67 per cent) were correctly predicted by roentgenography. Thirteen (33 per cent) were not diagnosed by roentgenography, but all 13 had significant roentgenographic lesions (figures 3 and 4). Of the 33 roentgenographic diagnoses of cavities, 26 (79 per cent) were verified by the pathologic findings. The 7 cases of false roentgenographic predictions, amounting to 21 per cent error, had multiple noncavitary lesions.

Parenchymal fibrosis was found in 54 (77 per cent) of the cases, but only 32 (59 per cent) were correctly diagnosed roentgenographically. Thirty-five roentgenographic diagnoses of parenchymal fibrosis were verified in the surgical specimens of 32 cases, a 91 per cent correlation.

TABLE 7

SEGMENTAL RESECTION FOR PULMONARY RESECTION IN 70 PATIENTS
Bacteriology of Surgical Specimens

	NEGATIVE		POSITIVE		END RESULTS		NUMBER OF CASES
	Smear*	Culture	Smear	Culture	Negative	Positive	
Patients with preoperative positive sputum	16	7	16	6	35	6	41‡
Patients with preoperative negative sputum†	12	17	6	2§	26	—	26

* Smear includes swabs of pus and homogenized lung tissue prepared with Ziehl-Neelsen stain.

† No positives on microscopy or culture of fortnightly to monthly sputum or gastric washings for six months before surgery.

‡ 3 additional patients died: one, six hours postoperatively, hemorrhage and excessive shock; one, five and one-half months postoperatively with meningitis, sputum negative for *M. tuberculosis*; one, two days postoperatively, acute cor pulmonale. Also 4 persistently infectious patients had disease on contralateral side, except one who had an ipsilateral spread.

§ The 2 patients with infectious sputum had very little preoperative antimicrobial treatment.

Atelectasis and bronchiectasis were rarely diagnosed from preoperative roentgenograms. However, these frequently were found on pathologic examination of the surgical specimen.

Bacteriologic findings: The bacteriologic findings are shown in table 7. In 26 patients who had sputum negative for tubercle bacilli for a period of six months or more before surgery, 6 had acid-fast bacilli on microscopy of the resected specimens, and 2 had culture positive for tubercle bacilli. These 2 cultures from surgical specimens were from patients who had had very little preoperative antimicrobial therapy. Of the group of 26 preoperative noninfectious patients, 12 specimens negative for acid-fast bacilli on microscopy and 17 negative for tubercle bacilli on culture were found. Moreover, all patients in this group of 26 remained noninfectious after segmental resections of their pulmonary disease even though in 3 cases there was accidental pleural contamination by caseous material.

In the group of 44 patients who had sputum positive for tubercle bacilli at the time of surgery, the specimens resected from 16 (36 per cent) were positive for acid-fast bacilli on microscopy and the specimens resected from 6 patients were positive for tubercle bacilli on culture. Resected specimens negative for acid-fast bacilli on microscopy were obtained from 16 patients and, from an additional 7, the specimens were negative for tubercle bacilli on culture.

In the study of end results (follow-up after surgery) of 41 patients, 35 (85 per cent) now have sputum negative for tubercle bacilli and 6 (15 per cent) are still discharging bacilli postoperatively. Of the 6 patients in this group who are still infectious, 4 had bilateral disease and one patient had an ipsilateral spread of disease before segmentectomy. It is interesting to note that, in the group of

patients who were noninfectious presurgically, microscopy of the specimens revealed acid-fast bacilli in 23 per cent and culture revealed tubercle bacilli in 8 per cent; while in the group of patients who were infectious at the time of surgery, the resected specimens were positive by microscopy in 36 per cent and by culture in 13 per cent.

DISCUSSION

It is apparent from the data presented in this paper that a long preoperative duration of disease is not in itself a deterrent to success in segmental surgery. The pulmonary tuberculosis usually remains localized in a segmental fashion. This allows the disease to be successfully managed by the segmental type of resection, provided the host-parasite balance favors the host by suppression of detectable tubercle bacilli, either naturally or through the action of antimicrobial therapy.

It is also apparent that serial roentgenographic examination with lateral and anteroposterior planigrams are an important guide for the interpretation of most lesions amenable to segmental resection. Bronchograms with rapidly eliminated water-soluble contrast media should probably be utilized more often to aid in delineating bronchiectasis and segmental bronchial stenosis. However, the surgeon at operation is often faced with the decision to excise or leave significant lesions not detectable preoperatively by roentgenographic or clinical study.

The bacteriologic findings indicate that, among a group of patients with sputum negative for tubercle bacilli preoperatively, there are almost as many lesions containing acid-fast bacilli or culturable tubercle bacilli as among patients with sputum containing tubercle bacilli before operation. Antimicrobial suppression by streptomycin, PAS, and isoniazid, as well as factors related to caseous areas, probably prevent the true viability of the tubercle bacilli from being detected by the cultural methods now being used. Otherwise, there should be a higher percentage of positive bacteriologic findings in the surgical specimens of those patients with sputum or gastric washings positive for tubercle bacilli at the time of surgery.

Segmental resection techniques are accompanied by more complications than lobectomy and pneumonectomy, but even the serious complications can usually be overcome without jeopardizing the final result. The 3 deaths (early and late) would probably not have occurred if these patients had had thoracoplasty instead of segmental resection.

Maintenance of the noninfectious state postoperatively has occurred in all 26 patients with sputum negative for tubercle bacilli at time of surgery. This low relapse rate indicates a superior degree of therapeutic effectiveness of segmental resection over collapse measures, but follow-up observations for a longer period of time will be necessary to confirm this. Reversal of infectiousness postoperatively occurred in 85 per cent of the patients who were discharging tubercle bacilli at the time of operation. Only one late death has resulted to date during the four years since the first segmental resection.

Excisional therapy has been especially designed to overcome the tendencies

for pulmonary tuberculosis to recur after other forms of treatment, both medical and surgical.

Two problems concerning the place of surgical resection in chemotherapeutically suppressed residual caseous lesions are: Will tubercle bacilli reappear in the sputum if the caseous necrotic lesion is resected? Follow-up observation of the present series is too short for a positive answer, the longest being only four years since surgery. Nevertheless, at this point the evidence is in favor of the idea that segmental resection of chemotherapeutically suppressed residual caseous lesions will markedly decrease the relapse rate in those patients with sputum containing tubercle bacilli at the time of surgery. Of 41 such cases, the sputum has remained infectious postoperatively in only 6. In 5 of the 6 cases tuberculosis has been present in the opposite lung, and in one case an ipsilateral spread of disease had occurred. Hence, recurrence of continuation of the infectious state in these cases was caused by active disease other than that for which segmental resection was performed.

The second problem is how long should antimicrobial therapy be continued, both before and after surgical excision? At the present time, if a resectable segmental lesion has been suppressed with absence of tubercle bacilli from the sputum for six months and recurrence after drug therapy is deemed probable because of the original amount and character of disease, surgical excision is usually advised. More experience is necessary before the duration of post-resective chemotherapy can be accurately defined. In the present series, however, antimicrobial treatment has been discontinued after two to six weeks in the 26 patients who were noninfectious preoperatively, unless surgical complications warranted a more prolonged course up to four months. Antimicrobial therapy is being continued beyond four months in the 6 patients who have continued to discharge tubercle bacilli following surgery.[2]

Preoperative roentgenographic and bacteriologic examinations were in only fair agreement with the bacteriologic and pathologic findings in the surgical specimens. The indications and status of excisional therapy for suppressed caseous necrotic lesions should be made more secure by study of the degree of such correlation in a larger series of cases. The experience from the present series is such that it would seem warranted to continue critical investigations of segmental resection combined with antimicrobial treatment to be compared with the results obtained by nonresection measures augmented by prolonged antimicrobial therapy.

Acknowledgment

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AN ESTIMATION OF THE EFFECTIVE ALVEOLAR RESPIRATORY SURFACE AND OTHER PULMONARY PROPERTIES IN NORMAL PERSONS[1]

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INTRODUCTION

Marie Krogh showed that a small amount of carbon monoxide can be used effectively as a tracer to study the diffusion of gas from the pulmonary alveolar space to the blood in the capillaries (1). Her results concerning diffusion of the indifferent gas, carbon monoxide, have been confirmed and it appears that gas diffusion across the pulmonary alveolar structures into the capillary blood is independent of pulmonary volume up to a certain point. When the volume becomes larger, beyond that point, the rate of carbon monoxide diffusion increases significantly.

The validity of the method was tested in normal men before attempting to apply it to patients with pulmonary pathologic states. The results are used further in the present study to obtain a more detailed interpretation and evaluation of gas diffusion across the alveolar-capillary membrane in normal man.

METHOD

The lung volume and its subdivisions were obtained in the sitting position by the method of Cournand and his associates (2). The intrapulmonary mixing index was measured by determining the per cent of nitrogen retained in the alveoli at the end of a seven-minute period of breathing 100 per cent oxygen (2).

To minimize the effect of unequal pulmonary alveolar ventilation on intrapulmonary carbon monoxide mixing, subjects were eliminated who had nitrogen retention greater than 2.5 per cent after breathing 100 per cent oxygen for seven minutes.

Approximately 100 cc. of 100 per cent carbon monoxide were mixed with 10 liters of room air in a recording spirometer. The spirometer was attached to a Cournand five-way respiratory valve. A 5-liter rubber bag was attached to the valve systems to facilitate thorough mixing of the carbon monoxide and room air in the spirometer.

Each of 12 normal persons was placed in the sitting position with the lips closed around the mouthpiece of the five-way respiratory valve. Each one was asked to exhale maximally into the room air, after which the subject inspired maximally from the recording spirometer. The subject exhaled quickly one-half of the inspired gas containing carbon monoxide, at which time the valve was turned to stop the gas flow in the system. A sample of this expired gas was collected and analyzed for carbon monoxide according to the method of Roughton and Root (3a). After a period of six seconds the patient exhaled maximally and rapidly the remaining gas in the lungs. A second sample of gas was collected in a pre-evacuated gas sampling bottle and analyzed for the per cent of carbon monoxide.

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In developing the interpretation of the experiments, it is assumed that the rate of carbon monoxide uptake is diffusion-controlled, and later an estimate is given to show that the resulting error is no more than 10 per cent. It is also assumed that the cross-sectional area involved in diffusion is less than the anatomic membrane area, whatever the size (molecular or greater) of the "interstices" through which the diffusion occurs. This is consistent with the findings of Shuler, Dames, and Laidler (4), who reported the total area of collodion membranes to be approximately ten thousand times the interstitial area.

A mathematical expression describing the effective alveolar surface (pores or spaces) may be developed as the following:

Let A = interstitial area or average cross-sectional area involved in diffusion, m^2
T = membrane thickness or average diffusion distance, μ
$q(t)$ = per cent of carbon monoxide in lung gas at time t (sec.)
R = A/T, m^2/μ,
α = absorption coefficient of carbon monoxide in plasma at 37°C.,
D = diffusion coefficient of carbon monoxide in plasma at 37°C., $cm.^2/sec.$,
V = lung volume (during the experiment) reduced to standard temperature pressure, liters
p = atmospheric pressure, mm. of mercury.

Then, according to the well-known Fick's law of diffusion,

$$-V\frac{dq}{dt} = (10^3 R\alpha D_p/760)\cdot q$$

so that

$$R = \frac{760V(2.30)}{10^3\alpha D_p}\cdot(\log_{10} q_1 - \log_{10} q_2)/(t_2 - t_1) \qquad (I)$$

According to O'Brien and Parker (5), $\alpha = 0.0143$ in human plasma at 37°C. For D, on the other hand, no measurement has yet been published, but it can be estimated as follows: A. Krogh (6) gave results showing that D for carbon monoxide is approximately 0.70 times that for oxygen, which is approximately 5.2×10^{-5} at 37°C. in water, according to an extrapolation of the measurements of Tammann and Jessen (7) at somewhat lower temperatures. Longmuir and Roughton (8) showed that D for CO is about inversely proportional to the viscosity of the fluid (in hemoglobin solutions), and the measurements of Bingham and Roepke (9) show that normal human plasma is approximately 2.05 times as viscous as water at 37°C. As a final estimate for D, these values give

$$D = 5.2 \times 10^{-5} \times (0.7/2.05) = 1.77 \times 10^{-5}.$$

With the above values for α and D, equation (I) becomes

$$R = 6.9 \times 10^4(V/p) \qquad ((\log_{10} q_1 - \log_{10} q_2)/(t_2 - t_1)). \qquad (II)$$

In terms of M. Krogh's d_{CO} (1),

$$R = 0.50\, d_{CO}. \qquad (III)$$

Actually, $R = A/T$ may be a simplified representation for a combination of two successive membrane layers having $R_1 = A_1/T_1$ and $R_2 = A_2/T_2$, respectively. It can readily be shown that, if this is so, then

$$1/R = 1/R_1 + 1/R_2. \qquad (IV)$$

However, it is not yet possible to measure R_1 and R_2 separately, assuming that these refer to alveolar and capillary membranes.

The ratio R between the average area and thickness of the respiratory membrane is a physiologic characteristic of the individual's lungs. This conclusion is supported by

Harrop's observation (10) that R and the susceptibility to mountain sickness are inversely related, and also that R is independent of altitude.

To estimate the error of assuming that the gas transfer is diffusion-controlled, one refers to Roughton's values (11) for the rate of the reaction:

$$Hb + CO \longrightarrow Hb \cdot CO. \qquad (V)$$

By applying the Arrhenius equation (12) to Roughton's rate values, it is found that the reaction of equation (V) has an experimental activation energy of approximately 12 kilocalories per mole and a rate constant of approximately 900 liters per millimole per second at 37°C. If half a liter of blood passes through the lungs during the experiment, if $R = 20$, and if the blood in the lung capillaries contains an average of 1 gm. of reduced hemoglobin per 100 cc., then Roughton's rate constant implies that less than 10 per cent error is caused by assuming that the gas transfer is diffusion-controlled and that the carbon monoxide tension is negligible within the capillaries.

RESULTS

Nitrogen retention in the pulmonary alveoli, after breathing 100 per cent oxygen for seven minutes, was less than 2.0 per cent in all normal persons. The mean pulmonary residual volume was 1,682 cc. in the sitting position. The normal range of R was 15 to 31 square meters of area per micron of thickness of functional respiratory membrane. The mean R for 12 normal persons was 20. There was no correlation between R and the pulmonary volume in these cases; the range of volume is listed in table 1. The value for R did not increase with lung

TABLE 1

SQUARE METERS OF PULMONARY ALVEOLAR SURFACE PER MICRON OF THICKNESS OF THE RESPIRATORY MEMBRANE (M^2/μ) AND KROGH'S DIFFUSION CONSTANT K AND d_{CO}

SUBJECT	BODY SURFACE	SITTING PULMONARY RESIDUAL VOLUME*	ALVEOLAR NITROGEN RETENTION	PULMONARY VOLUME DURING CARBON MONOXIDE DIFFUSION PERIOD*	K	d_{CO}	R M^2/μ	BAROMETRIC PRESSURE†
	m^2	*cc.*	*per cent*	*cc.*				*mm. Hg.*
1. R. G.	1.90	1,315	1.02	2,892	7.12	30.0	15	687.2
2. E. R.	2.05	1,190	0.95	3,357	6.35	30.9	15	690.6
3. R. J.	1.95	1,139	1.28	2,816	7.61	31.1	16	689.6
4. F. M.	1.70	1,399	1.34	2,800	8.16	33.1	17	689.8
5. H. W.	1.79	2,171	1.50	3,686	6.58	35.1	18	690.8
6. R. E.	1.82	1,609	1.31	3,278	7.65	36.2	18	691.8
7. R. S.	1.78	952	0.92	2,952	9.28	39.3	20	696.7
8. D. F.	1.97	1,721	1.11	3,728	7.73	41.9	21	688.0
9. J. H.	1.94	1,951	0.87	3,691	8.25	44.0	22	692.1
10. W. S.	1.96	3,235	1.09	5,243	5.81	44.1	22	690.6
11. L. W.	1.79	1,872	1.47	3,845	8.28	46.1	23	691.0
12. R. W.	1.90	1,625	1.27	3,247	13.11	61.6	31	691.6
Mean values		1,682					20	

* Standard temperature pressure dry.

† Minus water vapor pressure.

expansion until the lungs were filled beyond two-thirds of their capacity. The value of 15 to 31 square meters of functional pulmonary respiratory surface is approximately one-fourth of the corresponding anatomic area if the membrane thickness is 1 micron. A. Krogh (14) reported values that imply corresponding ratios of 0.4 for muscle, one-third for connective tissue, and one-fifth for india rubber.

To relate the present results for R to oxygen respiration it is first necessary to use metabolic rates to estimate the normal rate of oxygen uptake. The average man requires approximately 2,500 kilocalories of food energy and 22 moles, or 700 gm., of oxygen during a day of sedentary occupation. This necessitates the equivalent of a 4.5 per cent change in oxygen content of 13 kiloliters of gas or an oxygen uptake of approximately 0.25 millimoles per second. With this value, and assuming that $R = 20$ and $(D\alpha)_{O_2} = 4.7 \times 10^{-7}$ cm²/sec., it can be calculated (from Fick's law of diffusion) that the average oxygen pressure is 4 mm. of mercury less in the alveolar capillaries than in the alveolar air. This is an average for all of the blood in the capillaries between the pulmonary artery and the pulmonary vein—in the flow-wise sense. According to A. Krogh, the diffusions of carbon monoxide and oxygen across the alveolar membrane are physical processes related to molecular weight and tension. Thus, there are small differences between the diffusion characteristics of oxygen and carbon monoxide. Using the known values for oxygen diffusion and on chemical reactivity with hemoglobin, the following may be calculated.

The volume rate of flow in the capillaries can be calculated: if Q is the volume of blood pouring through the pulmonary capillaries each second, and if it has $(0.98 - 0.7) \times (0.15/16{,}800)$ moles of hemoglobin oxygenated in each cubic centimeter of this blood, then $Q = 2.5 \times 10^{-4}/(0.98 - 0.7) \times (0.15/16{,}800)$ is approximately 100 cc. Thus, the blood flow through the alveolar capillaries is approximately 100 cc. per second at rest.

It is also possible to estimate both the velocity of the capillary blood flow and the average time of blood passage through the lung capillaries. These estimates are based on available histologic and anatomic data which determine the amount of blood in the capillary network. The capillary inside diameter is approximately 8 microns (16), and much of the capillary network is exposed to air on two sides (16). Moreover, approximately seven-tenths of the aerated surface is the amount of the exposed capillary surfaces (16, p. 467). Hence, an aerated surface of 80 square meters corresponds to a capillary bed covering approximately 40 square meters ($80 \times 0.7 \times 0.7$, or approximately 40), which allows for $(1.0 - 0.7)/0.7$ or approximately half of the capillaries being exposed on both sides. The thickness of the capillary walls is approximately 1 micron (17), and the inside height of an equivalent rectangular cross-section is $\pi \times 8^2/4 \times 8$, or approximately 6.3 microns. As a final result then, the lung capillaries are estimated to contain approximately $40 \times 10^4 \times 6.3 \times 10^{-4} \times 8/(8 + 2) = 200$ cc. A flow of 100 cc. per second and a capacity of 200 cc. imply that two seconds is the average time in which the blood traverses the capillary network.

The preceding results can be combined with the well-known data on the equilib-

rium between oxygen and hemoglobin to estimate the rate of the erythrocyte's oxygenation during its traversal of the pulmonary capillaries. The equilibrium data show that an alveolar oxygen pressure of 105 mm. of mercury would be in equilibrium with hemoglobin which is approximately 99.3 per cent saturated. The rate of approach to this equilibrium can be calculated from the above value for the average oxygen pressure difference across the membrane (4 mm. of mercury) and the approximately two-second traversal time. If it be assumed that normal unoxygenated blood has an oxygen tension of 40 mm. of mercury and approaches the equilibrium pressure with a first order rate such that

$$p_{O_2} = 105 - (105 - 40)e^{-kt} = 105 - 65e^{-kt}$$

then,

$$4 = \int_0^2 65e^{-kt}\,dt/2 \qquad \text{and}$$

$$k = 65/2 \times 4 = 65/8, \text{ approximately.}$$

This corresponds to an approach to equilibrium with a half-time of $(8/65) \times \log_e 2 = 0.085$ second. Thus, in the first 0.085 second the oxygen pressure will rise to 72.5 mm. of mercury, which is equivalent to 94.6 per cent saturation of the hemoglobin. This leads to the conclusion, therefore, that in the normal person the blood traversing the pulmonary capillaries will have ample time to come practically to equilibrium with the alveolar oxygen (and become 99.3 per cent oxygenated when the alveolar oxygen pressure is 105 mm. of mercury). Similarly, it can also be concluded that, even in a person whose R value is only one-eighth of normal, the blood will be almost equilibrated (more than 99 per cent oxygenated at an alveolar oxygen pressure of 105 mm. of mercury) in the pulmonary capillaries.

This conclusion makes it possible to use a very simple expression to calculate the fraction of blood traversing the lungs which does not pass through the oxygenating capillaries. Let f be this fraction, let A_o be the per cent oxygenation of the arterial blood (oxygenated), and let V_o be the per cent oxygenation of venous blood. Then, if equilibrium is at E_o per cent oxygenation,

$$V_o f + E_o (1 - f) = A_o \qquad \text{or}$$
$$f = (E_o - A_o)/(E_o - V_o). \qquad (VI)$$

In a normal person having $A_o = 99$, $V_o = 72$, and $E_o = 99.3$, equation (VI) shows f to be 0.011, or approximately 1 per cent.

DISCUSSION

The passage of oxygen, nitrogen, carbon dioxide (and carbon monoxide as used in the experiment) from the pulmonary alveoli into the capillaries takes place by diffusion of the gases through either intercellular plasma or plasma in spaces between membrane molecules. Whatever the size of these spaces or "pores," they are plasma-filled interstices. It is correct to assume that modifications of (*a*) membrane thickness, (*b*) qualitative structural characteristics of the mem-

branes, (*c*) the vascular walls of the pulmonary alveoli, and (*d*) the viscosity of plasma all play a role in the rate of diffusion of gases from the alveoli into the pulmonary capillaries. In each individual pulmonary system the factors listed will cause variation of either the physiologic ratio $R = A/T$ or the diffusion coefficient D and thus affect the transfer of all gases across the pulmonary alveolar walls.

From the data reported it was calculated that the average oxygen tension in the capillaries of normal subjects was 4 mm. of mercury less than in the pulmonary alveoli. A pulmonary alveolar-capillary blood oxygen tension difference of 11 mm. of mercury was measured by Wilson and associates (17) and 10 mm. of mercury by Berggren (18). The 4 mm. of mercury value calculated from the data reported is more accurate than those reported by either Wilson and associates (17) or Berggren (18). The average oxygen tension in the capillary blood is a time average and, in order that the hemoglobin be almost completely oxygenated in its transit through the capillary network, it is necessary that the oxygen pressure of the capillary blood be high during most of the time the blood is passing through those capillaries.

The average value of $R = A/T = 20$, as measured in the 12 normal subjects, is substantiated by the results of the calculated alveolar-oxygen tension difference which falls well within the measured values reported (17, 18, 19).

The finding that R did not increase with lung expansion until the lungs were filled to at least two-thirds of their capacity suggests that both area A and thickness T are essentially constant, separately, in the range of lesser expansion. This phenomenon reflects the interplay between the areas of the capillary walls and the alveolar lining (13).

The assumption is made in this study that, following a single inspiration of carbon monoxide, all pulmonary alveoli may not contain exactly the same concentration of gas, but that distribution is uniform enough and diffusion is compensating enough to cause the calculated result to be a reliable physiologic $R = A/T$. According to A. Krogh and Lindhard (20), the alveolar air becomes uniform in composition when a deep inspiration is made immediately after the deepest possible expiration, contrary to the results of later but less extensive experimental studies (21). In the experimental subjects listed in table 1, the Cournand intrapulmonary mixing index indicates normal pulmonary mixing.

SUMMARY

Tests on the rate of uptake of trace amounts of carbon monoxide in the lungs are reinterpreted. The ratio of the average cross-sectional area involved in diffusion to thickness of the respiratory membranes in 12 normal men was found to be 15 to 31 meters per micron.

The calculated average pulmonary alveolar-capillary blood oxygen tension difference was 4 mm. of mercury.

Reasonable values of metabolism and histologic and gross anatomic quantities were used to estimate that the average aeration time of the blood is two seconds.

Other associated estimates are an alveolar-capillary capacity of 200 cc. and a flow of 100 cc. per second.

Blood oxygenation was described as increasing rapidly and practically to equilibrium with passage through the alveolar capillaries.

A simple expression is given for the fraction of blood passing through the lung which does not traverse the oxygenating capillaries. In normal man this fraction is approximately 0.01.

SUMARIO

Cálculo de la Superficie Respiratoria Alveolar Eficaz y de Otras Propiedades Pulmonares en las Personas Normales

Re-interprétanse aquí ensayos relativos a la velocidad con que se absorben en los pulmones cantidades en indicios de monóxido de carbono. La proporción de la zona transversal media comprendida en la difusión en relación con el espesor de las membranas respiratorias en 12 varones normales resultó ser de 15 a 31 metros por micra.

La diferencia en la tensión media calculada del oxígeno en la sangre de los capilares alvéolo-pulmonares representó 4 mm. de mercurio.

Usáronse valores razonables del metabolismo y de las cantidades histológicas y macroanatómicas para calcular que el tiempo medio de aereación de la sangre es de dos segundos.

Otros cálculos asociados son una capacidad capilo-alveolar de 200 c.c. y una corriente de 100 c.c. por segundo.

Según se describe, la oxigenación de la sangre aumenta rápidamente y alcanza prácticamente el equilibrio al pasar por los capilares alveolares.

Ofrécese una expresión simple para denotar la fracción de sangre que atraviesa el pulmón sin cruzar los capilares de oxigenación. En los sujetos normales, esa fracción es aproximadamente 0.01.

RESUME

Une évaluation de la surface respiratoire utile et autres propriétés du tissu pulmonaire chez les sujets normaux

Les auteurs fournissent une nouvelle interprétation des tests sur la vitesse d'absorption de traces d'oxyde de carbone dans les poumons. Le rapport trouvé entre l'aire transversale moyenne intéressée dans la diffusion et l'épaisseur de la membrane respiratoire, chez 12 hommes normaux, était de 15 à 31 mètres par micron.

La détermination de la différence moyenne de la tension de l'oxygène sanguin alvéolo-capillaire était de 4mm de Hg.

L'adoption de chiffres rationnels pour les valeurs se rapportant au métabolisme, à l'histologie et à l'anatomie, a permis d'évaluer à deux secondes le temps moyen d'aération du sang.

Des évaluations associées donnent une capacité alvéolo-capillaire égale à 200 cm³ et un débit de 100 cm³/sec.

L'oxygénation du sang est décrite comme un phénomène croissant rapidement et atteignant pratiquement l'équilibre dans la traversée alvéolo-capillaire.

Une formule simple exprime la fraction du sang passant dans le poumon sans traverser les capillaires d'oxygénation. Chez l'homme normal, cette fraction est évaluée approximativement à 0.01.

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ADMISSIONS TO GENERAL HOSPITALS[1,2]

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INTRODUCTION

Economics is the science of the production, distribution, and consumption of wealth; in any consideration of the "economics of case finding," our efforts to transmute wealth into the health of the populace should be analyzed. It is appropriate, therefore, to examine the chest roentgenographic unit of a general hospital to see how it *produces* health, i.e., results in the early detection of cases of tuberculosis; how it *distributes* health, i.e., makes it possible for case finding quickly to lead to proper therapy; and, finally, how efficiently it accomplishes these two objectives in terms of dollars as well as in man-hours of human endeavor.

MATERIALS AND METHODS

The case-finding roentgenographic unit under consideration functions in the Charity Hospital of Louisiana at New Orleans, which is a large general hospital with approximately 2,800 of its 3,200 beds in daily use. On a given day, one can see there almost any type of human ailment. Patients are attracted from all over the state, and the hospital maintains a very active emergency service. Charity Hospital has no connection with the Louisiana Department of Health and operates on its own budget as an autonomous state agency. Control over patients varies from excellent to poor.

With but few exceptions, all roentgenograms were interpreted by one reader (S. J.). Films are interpreted regularly on Mondays, Wednesdays, and Fridays. Immediately after the interpretations have been recorded and tabulated, clerks carry the reports to the respective wards for the inpatients' charts and to the record library for outpatients' charts. When tuberculosis is suspected, a copy of the report with the 70 mm. film attached is sent (usually within forty-eight hours) to the health officer of the respective parish, and a second copy goes to the State tuberculosis control officer. Many persons come to the hospital as visitors or for emergency treatment and may not be registered either as outpatients or inpatients; if they come from distant rural areas, much time may be consumed in follow-up studies. The Charity Hospital is an enormously popular place to which people turn when in trouble; this is reflected in the fact that more than one-half of the newly found cases of tuberculosis each year in New Orleans represent people who reported to the Charity Hospital for some other type of medical service only to learn of their tuberculosis by routine photofluorography.

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[2] Presented in part at the symposium on the *Economics of Case Finding* at the annual meeting of the National Tuberculosis Association, Los Angeles, California, May 19, 1953.

This chest roentgenographic unit is operated by the Division of Tuberculosis Control of the Louisiana Department of Health and has no administrative connection with the Charity Hospital. Such autonomy gives it an advantage over those general hospital case-finding centers which function as adjuncts to the usual radiologic services and are apt to suffer during periods of great budgetary and manpower drains. The personnel and the budget of the unit are committed to "screening" for tuberculosis through the taking of chest roentgenograms. It has neither responsibility nor control over follow-up procedures. The process of bringing tuberculosis suspects through confirmatory studies must be undertaken by health officers in the respective parishes or by physicians in charge of whichever ward receives the patient.

PLAN OF STUDY

The limits of usefulness and economic costs of the chest roentgenographic unit of a large general hospital in case finding have been studied. Much has been written about case finding, and the general principles have been accepted. Nevertheless, the relative values of feasible procedures have seldom been plainly stated. It is certain that there are many ways of finding tuberculosis in a person who does not regard himself as sick enough to consult a physician.

In an attempt to learn the effectiveness of the chest roentgenographic unit of this hospital, all films taken were analyzed to find out:

1. The yield of tuberculosis suspects and of active tuberculosis of the lungs
2. The yield of significant nontuberculous disease
3. The price of a single frame of 70 mm. film as an index of the expense of the procedure
4. The cost of finding a single case of active pulmonary tuberculosis
5. The inherent strengths and weaknesses of this method of case finding
6. The ability of this approach to suggest the true incidence of tuberculosis in a community by detecting previously unknown instances of active pulmonary tuberculosis
7. The speed with which suspects are given the benefit of supplementary 14- by 17-inch film studies.

All interpretations were tabulated conventionally into *tuberculosis suspects*, *nontuberculous disease*, *technically unsatisfactory*, and *normal*.

It is always desirable to find out what ultimately happens to the person whose photofluorographic image is interpreted to indicate pulmonary tuberculosis. Because the chest roentgenographic unit has no responsibility for follow-up procedures, it was necessary to rely on hospital records and on case-registry data throughout the state of Louisiana for this information.

OBSERVATIONS

From the time that the 70 mm. equipment was installed (January 2, 1946) until March 1, 1953, 428,254 exposures were made. As figure 1 indicates, 12,261 films (2.9 per cent) were technically unsatisfactory; 13,602 (3.1 per cent) were classified *tuberculosis suspects*, the chief reason for the existence of this unit; 21,500 (5 per cent), were regarded indicative of *nontuberculous disease*, chiefly

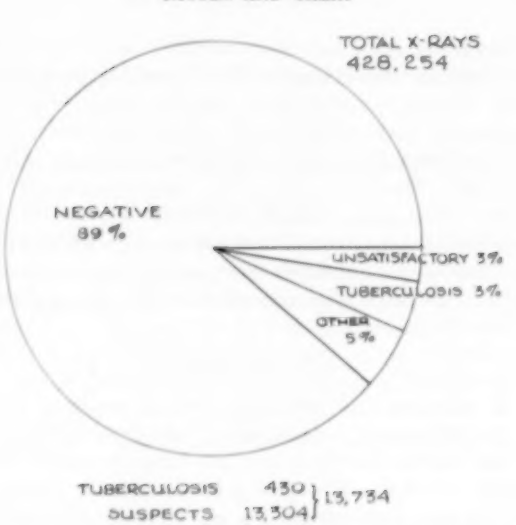

FIG. 1. Photofluorograms from the Charity Hospital Chest X-Ray Unit (1946–1953)

cardiac abnormalities; and 380,766 (89 per cent) were interpreted as *normal*. To determine the value of the unit in discovering those with tuberculosis who might not otherwise be detected for a long time, the 13,602 suspects were studied to determine how many of them had clinically important tuberculosis.

Tuberculosis: Of the 13,602 suspects, clinical studies of 6,617 were carried through to the point of establishing a definitive diagnosis. It is disappointing that the remainder could not be traced, but it is possible that many of them reported elsewhere for confirmatory studies. One of every 3 suspects so examined was found to have active tuberculosis. This means that, for every 1,000 photofluorograms, 4.3 active cases of tuberculosis were revealed. After removing from the study those 488 persons known to have *tuberculosis*, to be *tuberculosis contacts*, or *suspects* because of significant pulmonary symptoms, there remained 1,903 patients, or approximately 4.4 in each 1,000, who represented new cases of tuberculosis not likely to be brought to light by any other form of survey. These were people who had come to the hospital for some entirely unrelated illness, only to be found to have clinically active tuberculosis.

Nontuberculous diseases: As may be seen in figure 1, 21,500, or approximately 5 per cent of the 428,254 photofluorograms, were interpreted as indicating *nontuberculous disease* which justified clinical study. Approximately 3.7 per cent of all persons examined were thought to have some cardiovascular abnormality. The estimation of cardiac enlargement was always a difficult matter. Frequently the extreme disproportion between the size of the heart and the thorax made the diagnosis simple. In less obvious cases, however, the decision was made by adopting the principle of "over-reading", i.e., classifying as enlarged any heart with total transverse diameters which apparently exceeded 50 per cent of the

transverse thoracic diameter. Other more accurate methods were considered but found impracticable in this unit.

The 2,449 films obtained in 1952 and classified by this method as indicating cardiac enlargement were reviewed by the cardiologist in charge (Dr. Louis Levy, II). He rejected approximately 5 per cent as not indicating cardiac enlargement, found that 78 per cent of the patients were currently being studied for cardiac disease in one of the clinical divisions of the hospital, and directed the clinical study of the remainder. Of the 397 so studied, 99 (25 per cent) were found not to have any clinical evidences of cardiac disease. For the most part they were either children or pregnant women, two groups of persons in whom estimation of cardiac enlargement from photofluorography is particularly difficult. Cardiac disease was detected in the remaining 298 patients when it had not been known prior to their coming to the hospital. This represents a yield of approximately 4 cases per thousand persons examined routinely.

Approximately 1.3 per cent of all persons examined had evidences of some nontuberculous pulmonary disease of significance. These instances have not been tabulated, but it is known that a number of cases of bronchogenic carcinoma owe their early diagnosis to such routine examination.

DISCUSSION

Routine photoradiography of presumably nontuberculous healthy persons is an accepted method of finding unsuspected tuberculosis. The same method is also of value in finding nontuberculous intrathoracic disease. The present study suggests that the routine roentgenographic examination of the chest, as would be naturally inferred, uncovers more tuberculosis among sick persons than among healthy ones. A consideration of the economics of case finding is concerned with costs and values, i.e., in obtaining the largest possible number of tuberculosis suspects for the smallest possible expenditure of money and human endeavor. The best method of conducting such surveys is still to be determined. Possibly, no one method will ever be universally satisfactory. Routine roentgenographic examination of the chest for each person who enters a general hospital has certain features which merit study.

One important feature is accessibility. The unit in question is in the basement of the hospital, one floor below the admitting room. This physical disadvantage has been reduced by obtaining an excellent degree of cooperation from the hospital admitting officers who refuse to examine a single patient (except those in grave emergency) until after he has gone to the basement for a chest film. A certain proportion of patients are admitted at night or are too sick to stand erect for the examination; most of their roentgenograms are obtained in the main radiology units of the hospital. Experience has shown that nothing maintains the steady flow of patients through the unit so much as keeping the residents and interns interested in the program.

The second important feature with respect to general hospital survey units has to do with their higher yield of tuberculosis. With 428,254 photofluorograms, 2,391 cases of tuberculosis were found, 1,903 of which had not previously been

detected. This was the yield despite the fact that approximately 7,000 suspects eluded follow-up procedures, chiefly by returning to rural areas before confirmatory studies could be completed. As the suspicion of tuberculosis was verified in one of each 3 instances, it is probable that more than 2,500 persons with tuberculosis in these seven years passed before the photofluorographic screen but were not apprehended for study. Presumably the maximal yield should have been not 4 but 10 active cases of tuberculosis per 1,000 frames of 70 mm. film. It would be highly instructive to compare this group of "new" cases with a similar group detected by other approaches to case finding.

A third feature is the question of economy. It costs 29.8 cents to take a single frame of 70 mm. film (2). This figure, confirmed by computations of cost accountants of the Public Health Service, is lower than any other published cost. In a general hospital chest roentgenographic unit, the costs are easily tabulated; this is not always true for other methods of case finding. Frequently Christmas Seal Sale funds are expended by a local or constitutent association in pre-survey education and are not tabulated as expenditures for the program. No one can deny the great need for such effort if the survey is to succeed; yet there is almost no information to determine its cost.

Whatever that cost may be, computed in cents per frame of 70 mm. film, it does not apply to a survey of the type of the present case. This advantage is indigenous to the tax-supported hospital and is maximal when the budget of the survey unit is independent of the budget of the radiology department of the hospital. A general hospital which serves private patients cannot provide this service at so low a figure, but still can provide routine chest roentgenograms at not much greater cost and with better diagnostic yield than for routine blood chemical or serologic tests.

It may be computed that it costs the state of Louisiana approximately $80 to detect each person with active tuberculosis among those treated at the hospital, or approximately $110 for each one not previously suspected. The same expenditure of money and effort also reveals persons with other intrathoracic abnormalities: at least 4 instances of previously unsuspected cardiac abnormalities and an undetermined number of cases of nontuberculous pulmonary disease. There is no way to determine what portion of this expenditure should be logically charged up to the discovery of these nontuberculous diseases.

A fourth feature of the general hospital type of survey is the speed of confirmation of the diagnosis. Delay in diagnosis is a long-recognized factor in permitting progression of pulmonary tuberculosis. Routine photofluorography has been regarded as the most effective means of preventing this delay; yet there is often a lapse of time between the taking of the photofluorogram and the taking of the definitive 14- by 17-inch film. It is not known how much time elapses in the ordinary survey between these two important landmarks in diagnosis; little has been published to portray usual experience. In an effort to determine whether the present chest roentgenographic unit was actually facilitating the clinical diagnosis of suspects, the period between taking the photofluorogram and of the larger film was studied. It will be seen (figure 2) that, when patients entered the hospital immediately after the photofluorographic examination, the delay

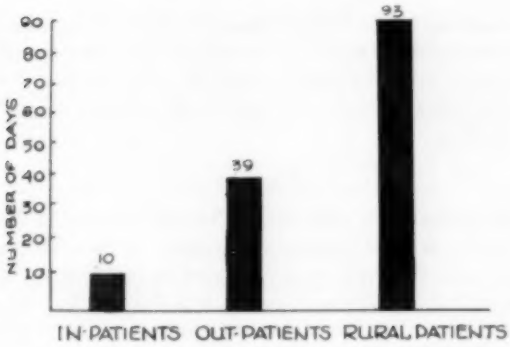

FIG. 2. Number of days which elapsed between taking of 70 mm. film and 14- by 17-inch film.

was minimal; when patients reported to the outpatient departments for clinical studies, the delay was moderate; when they returned home, the delay was maximal.

As reports of photofluorograms are usually on the charts of hospital inpatients within forty-eight hours of development, an average of eight days passed before the patient was sent for a larger film. Frequently this indicated that the patient had in the interim undergone a surgical operation, had become desperately ill, or otherwise was unable to be moved to the main radiology department for confirmatory studies. In other instances it meant that interns simply regarded the small film reports as of lesser importance than the original illness for which the patient entered the hospital. Such human inertia centers into any scheme for case finding. It is believed that the delay is potentially least of all when the survey film is taken at an institution wherein follow-up studies are possible. The survey team has no jurisdiction over the patients in the hospital; only by the careful and tedious process of education can this delay be further minimized. Too often the busy outpatient dispensary physician pays more attention to the patient's primary illness than he does to the report of the chest roentgenogram. This is one explanation for the "moderate" delay in clinical evaluation of outpatients. Much time is, of necessity, lost for rural patients because reports must be routed through understaffed local health departments.

One observation justified further investigation. Of the original group of *tuberculosis suscepts*, 636 were found by thorough clinical investigation to have inactive pulmonary tuberculosis, almost all minimal. Of the 1,903 cases of active pulmonary tuberculosis in persons who were entirely asymptomatic and previously unknown to have any pulmonary disease, only 18 per cent were minimal. Why had 82 per cent progressed without developing any symptoms usually ascribed to tuberculosis? This is in marked contrast to the findings of other surveys in which the majority of cases of presymptomatic tuberculosis are found at the minimal stage. Further research is needed to determine the types of illness which brought these persons to seek medical attention.

Finally, it should be observed that the present study represents an attempt to provide specific data for comparison with other types of surveys. Much money has been expended in promoting many types of investigation. The time has now come to determine what has been received for the money, i.e., to study the economics of case finding.

SUMMARY

The chest roentgenographic unit at the Charity Hospital in New Orleans has demonstrated that a general hospital can engage in case finding quickly, economically, effectively. For each $110 expended in bringing to light a new case of active pulmonary tuberculosis, attention was directed to four previously unrecorded instances of cardiac disease and an untabulated number of nontuberculous pulmonary diseases.

The chest roentgenographic unit in a general hospital is essentially a screening device. Unlike other case-finding devices, it needs no educational effort to induce subjects to have chest roentgenograms. Metaphorically, it can point a finger at someone with clinical tuberculosis but cannot place the restraining hand on him. Its usefulness is therefore directly proportionate to the thoroughness of follow-up procedures.

The writers' experience causes them to reiterate the familiar admonition that, to protect the investment in case finding, expenditures for follow-up must be increased.

SUMARIO

Ingresos en los Hospitales Generales

La sección de radiografía torácica del Hospital Charity de Nueva Orleans ha demostrado que un hospital general puede emprender el descubrimiento de casos en forma rápida, económica y eficaz. Por cada $110.00 gastados en poner de manifiesto un nuevo caso de tuberculosis pulmonar activa, se enfocó la atención en cuatro casos no inscritos antes de afección cardíaca y en un número intabulado de neumopatías no tuberculosas.

La sección de radiografía torácica de un hospital general viene a ser en el fondo un medio de despistaje. En contraposición a otros medios de descubrimiento de casos, no requiere ningún esfuerzo educativo para persuadir a los clientes a que se dejen hacer exámenes radiográficos del tórax. Metafóricamente, puede apuntar con el dedo a alguien que tiene tuberculosis clínica, pero no puede echarle mano para detenerlo. Su utilidad se halla, pues, en razón directa a la eficacia que revistan los procedimientos de observación subsiguiente.

Las observaciones de los AA. los impulsan a reiterar la conocida amonestación de que, para resguardar lo invertido en el descubrimiento de casos, hay que aumentar lo gastado en la observación subsiguiente.

RESUME

Admissions dans les hôpitaux généraux

Le service de radiologie thoracique de "Charity Hospital," à la Nouvelle Orléans, a pu démontrer qu'un hôpital général pouvait se livrer au dépistage de la tuberculose avec rapidité, économie et efficacité. Pour chaque cent dollars dépensés pour découvrir un nouveau cas de tuberculose pulmonaire en activité, l'attention a été attirée sur quatre cardiopathies non enregistrées antérieurement et sur un nombre non enregistré d'affections pulmonaires non tuberculeuses.

Le service de radiologie thoracique d'un hôpital général est essentiellement un moyen de criblage, il n'est pas besoin d'efforts pour inciter les individus à accepter l'examen radiologique thoracique. Employant une métaphore, on peut dire de l'examen radiologique qu'il "montre du doigt le sujet atteint de tuberculose active, mais qu'il n'a pas le pouvoir de l'empoigner." Sa valeur est donc directement proportionnée au degré de perfection des procédés de surveillance des cas.

L'expérience des auteurs les incite à réitérer l'avertissement familier déclarant que, lorsqu'il s'agit de sauvegarder les fonds engagés pour le dépistage, il est nécessaire d'accroître les dépenses affectées à la surveillance des cas.

Acknowledgment

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THE RESISTANCE OF TUBERCLE BACILLI TO THE BACTERICIDAL ACTION OF BENZALKONIUM CHLORIDE (ZEPHIRAN®)[1]

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INTRODUCTION

Specimens of sputum to be examined by culture for tubercle bacilli are usually treated with an alkaline or an acid solution. This treatment has a dual function: First, it renders the sputum less viscous and more homogeneous, thus making it possible to manipulate the sample and to concentrate the bacteria in it by centrifugation; and second, this treatment is lethal to most of the nonacid-fast microorganisms and thus serves to "decontaminate" the specimen.

Dubos developed a method employing enzymes for sputum digestion (1), reasoning that the enzymatic procedure might be less injurious to tubercle bacilli than exposure to an acid or alkaline reaction. It has indeed been demonstrated that tubercle bacilli are not killed by exposure to mixed pancreatic enzymes (2, 3), but it is also apparent that many nonacid-fast microorganisms survive such treatment (4, 5). A major obstacle, then, to the development of enzymatic or other new methods for sputum digestion has been the inability to eliminate "contaminating" microorganisms. A simple procedure which may overcome this obstacle is outlined in the present communication.

OBSERVATIONS

Observations were made of the bactericidal activity of several disinfectants on tubercle bacilli and on a group of nonacid-fast bacteria.

Stock cultures of an attenuated strain of the bovine tubercle bacillus (BCG-Phipps) were maintained in standard Tween®-albumin liquid medium. Cultures of *Micrococcus pyogenes* var. *aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* type B were prepared in beef-heart infusion broth. Fully grown cultures of these microorganisms were diluted 1:100 into a sterile solution of 0.02 M mixed phosphate buffer at pH 7.4 containing 0.1 per cent bovine plasma fraction V and 0.02 per cent Tween® 80. These suspensions were then mixed with solutions in water of various concentrations of the disinfectants. After incubation for one hour at 38°C., each mixture was diluted 1:10 in fresh buffer containing albumin and Tween® 80, and an aliquot of this dilution was then placed on Dubos albumin-agar (for tubercle bacilli) or on beef-heart infusion agar (for nonacid-fast bacteria). Appropriate control tubes containing no disinfectant were included in each experiment. The lowest concentration of each disinfectant which produced sterilization of the bacterial suspensions as indicated by the absence of growth on the test plates is recorded in table 1.

As may be seen in table 1, all of the agents used had approximately the same bactericidal activity on the tubercle bacillus as on nonacid-fast bacteria with the notable exception of the cationic detergent benzalkonium chloride (alkyldimethylbenzylammonium chloride, Zephiran®). The tubercle bacillus showed a

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TABLE 1

THE COMPARATIVE EFFECT OF SEVERAL DISINFECTANTS ON TUBERCLE BACILLI AND ON NONACID-FAST BACTERIA

DISINFECTANT	LOWEST CONCENTRATION WHICH BROUGHT ABOUT STERILIZATION OF SUSPENSIONS OF THE FOLLOWING MICROORGANISMS UNDER THE CONDITIONS OUTLINED IN THE TEXT			
	Tubercle Bacilli	Nonacid-Fast Bacteria		
	BCG-Phipps	*Micrococcus pyogenes* var. *aureus*	*Escherichia coli*	*Klebsiella pneumoniae* type B
	per cent	*per cent*	*per cent*	*per cent*
Formaldehyde	0.05	0.05	0.05	0.025
Sodium hypochlorite	<0.05	<0.05	<0.05	<0.05
Potassium permanganate	0.005	>0.005	0.005	0.0025
Benzalkonium chloride	>0.1	0.005	0.005	0.0025
Acetone	25	25	25	25
Methyl alcohol	50	25	50	25
Propylene glycol	50	50	50	25

marked resistance to the lethal effect of this compound. Whereas a final concentration of 0.005 per cent benzalkonium chloride was sufficient to kill the nonacid-fast microorganisms, BCG-Phipps remained viable when exposed to a concentration of 0.1 per cent under these conditions.

Next, an experiment was designed to determine if similar differences existed in the susceptibility of mycobacteria and nonacid-fast microorganisms to the bacteriostatic action of benzalkonium chloride.

Various concentrations of benzalkonium chloride were added to Dubos liquid medium containing glucose and albumin but no Tween® 80. Beef-heart infusion broth was added to a final concentration of 5 per cent, thus producing a medium which supported well the growth of both tubercle bacilli and nonacid-fast bacteria. The tubes were inoculated by adding dilutions in water of the various microorganisms so as to give a final concentration of 10^{-3} of fully grown stock cultures. Tests performed at pH 6.5 and at pH 7.4 gave identical results.

As may be seen in table 2, the tubercle bacilli and the nonacid-fast bacteria were approximately equally susceptible to the bacteriostatic action of benzalkonium chloride, in contrast to the marked divergence observed in susceptibility to the bactericidal action of this substance.

Further tests were done to determine the resistance of various strains of mycobacteria to the germicidal action of benzalkonium chloride.

Fully grown stock cultures (nonacid-fast bacteria in beef-heart infusion broth, mycobacteria in Tween®-albumin medium) were diluted 1:100 in sterile 0.1 per cent bovine plasma albumin. After mixing, samples were removed and serial dilutions in 0.01 per cent bovine albumin were plated on Dubos albumin-agar or on beef-heart infusion agar in order to determine the approximate numbers of organisms in the initial bacterial suspension. Benzalkonium chloride was then added to each of the suspensions to a final concentration of 0.05 per cent. The tubes were maintained at room temperature and samples

TABLE 2

THE BACTERIOSTATIC ACTION OF BENZALKONIUM CHLORIDE ON VARIOUS MICROORGANISMS

FINAL CONCENTRATION OF BENZALKONIUM CHLORIDE IN THE MEDIUM	GROWTH OF MICROORGANISMS			
	Tubercle Bacilli	Nonacid-Fast Bacteria		
	BCG-Phipps	*Klebsiella pneumoniae* type A	*Micrococcus pyogenes* var. *aureus*	*Escherichia coli*
per cent				
0.02	0*	0†	0†	0†
0.002	0	0	1	1
0.0002	8	8	8	8
None	8	8	8	8

* Growth after incubation at 38°C. for two weeks, graded by visual observation from 0 (no growth) to 8 (dense growth).

† Growth after incubation at 38°C. for two days, graded as above.

were removed at intervals, diluted serially, and the numbers of surviving bacteria determined on appropriate medium as described above.

Another set of experiments was identical except that the bacterial suspension and the serial dilutions were in distilled water instead of albumin-water. In the aqueous system the pattern of bacterial survival on exposure to benzalkonium chloride for thirty minutes was the same as that obtained when using the albumin-water diluent.

The results presented in table 3 reveal that all of the forms of true tubercle bacilli tested were remarkably resistant to the bactericidal action of benzalkonium chloride. Human, bovine, and avian strains of tubercle bacilli, and virulent, attenuated, and avirulent forms of some of these strains all behaved essentially the same. In contrast, however, was the behavior of saprophytic mycobacteria, which, like the nonacid-fast bacteria, were rapidly killed by exposure to 0.05 per cent benzalkonium chloride.

These results suggested that benzalkonium chloride might be of practical value in eliminating "contaminating" microorganisms from specimens to be examined by culture for tubercle bacilli. Although no large-scale laboratory trial has yet been attempted, preliminary studies demonstrate the feasibility of such an application. In one of these experiments attenuated bovine tubercle bacilli (BCG-Phipps) were suspended in 0.02 M mixed phosphate buffer at pH 7.4 and in the same buffer containing a final concentration of 0.5 per cent bovine plasma albumin. By serial dilution and plating, counts were made of the numbers of viable organisms in these suspensions. To the suspensions were then added one-fifth of their volume of a saturated solution of Difco pangestin 1:75 in water (not sterilized). After incubation for two hours at 38°C., benzalkonium chloride was added to produce a final concentration of 0.05 per cent. After standing for thirty minutes at room temperature, the mixtures were placed in sterile tubes and centrifuged at high speed for twenty minutes. The supernatant solutions were removed aseptically and discarded. The sediments were suspended in the same volume and type of diluent as that used prior to the digestion, and the viable

TABLE 3

THE EFFECT OF BENZALKONIUM CHLORIDE ON THE VIABILITY OF NONACID-FAST BACTERIA, SAPROPHYTIC MYCOBACTERIA, AND TUBERCLE BACILLI

TEST CULTURE	APPROXIMATE NUMBERS OF VIABLE MICROORGANISMS PER ML. OF SUSPENSION AFTER EXPOSURE TO 0.05 PER CENT BENZALKONIUM CHLORIDE FOR:		
	0 hours	½ hour	4 hours
Micrococcus pyogenes var. *aureus* (nonacid-fast)	10^6	$<10^1$	$<10^1$
Proteus vulgaris (nonacid-fast)	10^6	$<10^1$	$<10^1$
Klebsiella pneumoniae, type B (nonacid-fast)	10^6	$<10^1$	$<10^1$
Mycobacterium phlei (saprophytic acid-fast)	5×10^5	$<10^1$	$<10^1$
Mycobacterium smegmatis (saprophytic acid-fast)	10^6	$<10^1$	$<10^1$
Mycobacterium ranae (saprophytic acid-fast)	10^6	$<10^1$	$<10^1$
Mycobacterium sigerson (avian acid-fast)	3×10^5	10^5	10^5
Mycobacterium H37Ra (human avirulent acid-fast)	10^6	5×10^4	5×10^4
Mycobacterium BCG-Tice (bovine attenuated acid-fast)	5×10^4	5×10^4	10^4
Mycobacterium BCG-Phipps (bovine attenuated acid-fast)	5×10^4	5×10^4	5×10^4
Mycobacterium H37Rv (human virulent acid-fast)	10^5	10^5	10^5
Mycobacterium Vallée (bovine virulent acid-fast)	2×10^5	2×10^5	2×10^5

bacteria were enumerated by serial dilution and plate counting. Pure cultures of tubercle bacilli were obtained, and the numbers were the same as those in the starting suspension. The results were essentially the same whether or not the suspending medium contained bovine albumin.

In another experiment a bacterial mixture consisting of one drop each of fully grown cultures of the attenuated tubercle bacillus BCG-Phipps, *Micrococcus pyogenes* var. *aureus*, *Klebsiella pneumoniae* type A, and *Proteus vulgaris* was made in 5 ml. of water and also in 5 ml. of beef-heart infusion broth. These mixed bacterial suspensions were then digested with pancreatic enzymes and treated with benzalkonium chloride in a fashion similar to that described above. No centrifugation was employed. After exposure to benzalkonium chloride for thirty minutes, samples were removed and placed on media suitable for the cultivation of tubercle bacilli and also on media appropriate for the various nonacid-fast bacteria. In all cases no growth resulted in beef-heart infusion media, and pure cultures of tubercle bacilli were obtained on Dubos albumin-agar, thus demonstrating that all bacteria except the tubercle bacilli had been killed.

DISCUSSION

The findings reported here indicate that tubercle bacilli were resistant to the bactericidal action of the quaternary ammonium germicide benzalkonium chloride. In contrast, several saprophytic mycobacteria and nonacid-fast bacteria were susceptible.

Previous workers have observed the effect of cationic detergents on tubercle bacilli. In fact, the monograph on quaternary ammonium germicides by Lawrence (6) contains a section devoted to the action of these compounds on mycobacteria. In this section there is discussion of experimental data from several reports (7–12) which, taken together, lead to conclusions in general agreement with those of the present investigation. In an additional recent study, it was also noted that surface-active agents exerted little lethal effect on tubercle bacilli (13). However, none of the previously published reports presents an analysis of the bactericidal action of benzalkonium chloride on various types of mycobacteria under one set of conditions. Perhaps this deficiency accounts, at least in part, for the lack of emphasis placed on these observations.

The resistance of tubercle bacilli to the lethal effects of benzalkonium chloride is of interest from both practical and theoretical points of view.

One possible practical application of this knowledge is the use of benzalkonium chloride in treating specimens sent to the laboratory for cultivation of tubercle bacilli. Many, or perhaps all "contaminating" nonacid-fast microorganisms could thus be eliminated from such specimens. In addition, on the basis of observations made thus far, it appears that this treatment would kill saprophytic mycobacteria, which are often a source of confusion, especially in specimens obtained from the gastrointestinal or urinary tracts.

Benzalkonium chloride treatment might also serve to distinguish saprophytes from avirulent or attenuated tubercle bacilli, a problem of current interest in connection with bacteriologic study of pathologic specimens from tuberculous patients (14). Saprophytic mycobacteria and true tubercle bacilli differ in many characteristics other than susceptibility to benzalkonium chloride. Among well-recognized differences between these groups of microorganisms are the differences in growth characteristics *in vitro* and *in vivo*, in cytochemical reactions such as the neutral red test, and in susceptibility to certain antimicrobial drugs. In many instances, exceptions have been found to rules formulated for distinguishing saprophytes from tubercle bacilli. Obviously, many more tests must be done before it is known whether or not all saprophytic mycobacteria are more susceptible than tubercle bacilli to the bactericidal action of quaternary ammonium germicides.

Studies done thus far are inadequate to justify the recommendation of a particular method for the use of benzalkonium chloride in treating specimens to be cultured for tubercle bacilli. Certain facts, however, should be borne in mind when employing this agent. As it does manifest a bacteriostatic action against tubercle bacilli, any treatment of sputum or other specimens with benzalkonium chloride should be followed by centrifugation, washing, or dilution to ensure that the concentration of the cationic detergent in the culture medium is below the bacteriostatic level. Also, when acid or alkaline digestion of sputum or gastric washings is used, benzalkonium chloride should be applied only after neutralization of the digested specimen, since it is known that the pH of the medium influences the bactericidal activity of quaternary ammonium compounds, this activity being increased by an alkaline and decreased by an acid environment (6).

Although not specifically investigated in the present work, it seems probable that benzalkonium chloride might be added to sputum along with the enzyme preparation when enzymatic digestion is used. The recent note of Saxholm (3) describes preliminary experiments in this field, using a different detergent.

Another possible use of benzalkonium chloride in the preparation of material for the cultivation of tubercle bacilli might be mentioned. A relatively new bacteriologic technique which may be of value in the tuberculosis laboratory is the collection of bacteria from a specimen by filtration through specially prepared paper discs, the so-called membrane or millipore filters (15, 16). Morning mouth washings, urine, cerebrospinal fluid, or other suitably filterable material might be treated with benzalkonium chloride and then passed through a membrane filter, a collection procedure which is likely to be much more efficient than centrifugation. The filter discs would then be incorporated in or impregnated with suitable medium and the bacteria on them permitted to grow into colonies on incubation.

As benzalkonium chloride in aqueous solution does not kill tubercle bacilli rapidly, obviously this preparation should not be used as a disinfectant in connection with the care of tuberculous patients or in the laboratory. It might be pointed out, however, that a recent report states that alcoholic solutions of a cationic detergent were rapidly bactericidal for tubercle bacilli (17).

From the theoretical viewpoint, the resistance of tubercle bacilli to the bactericidal action of benzalkonium chloride is also of interest. The most widely accepted hypothesis concerning the mechanism of action of cationic detergents on microorganisms is that these substances react with certain constituents of the bacterial cytoplasmic membrane and alter its permeability so as to permit penetration of the detergent, inactivation of certain enzymes, loss of vital intracellular components, and death of the bacterium (18–21). If this hypothesis is valid, then the resistance of tubercle bacilli to the action of benzalkonium chloride is most likely based on unique features of the cytoplasmic membrane in this group of microorganisms. No facts are at present available concerning the chemical nature, anatomic position, or physiologic functions of the plasma membrane of tubercle bacilli. In this connection, it is of some interest that, in addition to tubercle bacilli, bacterial and fungal spore forms are the only microorganisms recognized to be resistant to the quaternary ammonium germicides.

SUMMARY

Tubercle bacilli are not killed by exposure to moderate concentrations of the cationic detergent benzalkonium chloride (Zephiran®), while even low concentrations of this substance exert a rapid bactericidal action on several saprophytic mycobacteria and nonacid-fast microorganisms.

Tubercle bacilli, saprophytic mycobacteria, and certain nonacid-fast microorganisms are approximately equally susceptible to the bacteriostatic action of benzalkonium chloride.

These observations suggest the possible use of this quaternary ammonium germicide to eliminate "contaminating" nonacid-fast microorganisms and saprophytic mycobacteria from specimens to be examined by culture for tubercle

bacilli. They also indicate that aqueous solutions of benzalkonium chloride are not suitable for disinfecting material likely to contain tubercle bacilli.

SUMARIO

La Resistencia de los Bacilos Tuberculosos a la Acción Bactericida del Cloruro de Benzalconio (Zefirán)

A los bacilos tuberculosos no los mata la exposición a concentraciones moderadas del detergente catiónico cloruro de benzalconio (Zefirán), mientras que hasta concentraciones bajas de esa substancia ejercen rápido efecto bactericida sobre varias micobacterias saprófitas y microbios anácidorresistentes.

Los bacilos tuberculosos, las micobacterias saprófitas y ciertos microbios anácidorresistentes son más o menos igualmente susceptibles al efecto bacteriostático del cloruro de benzalconio.

Sugieren esas observaciones el posible uso de este cuaternario amónico germicida para eliminar los "contaminantes" microbios anácidorresistentes y micobacterias saprófitas de los ejemplares que van a examinarse en cultivos en busca de bacilos tuberculosos. Indican además que las soluciones acuosas de cloruro de benzalconio no se prestan para desinfectar substancias susceptibles de contener bacilos tuberculosos.

RESUME

Résistance du bacille tuberculeux à l'action bactéricide du chlorure de benzalconium (Zéphiran)

Les bacilles tuberculeux ne sont pas tués par l'exposition au chlorure de benzalconium (Zéphiran), détersif cationique, en concentration moyennes alors que même à faible concentration ce composé exerce una action bactéricide sur un certain nombre de mycobactéries saprophytes et de germes non acido-résistants.

Les bacilles tuberculeux, mycobactéries saprophytes et certains germes non acido-résistants ont une sensibilité approximativement égale à l'égard de l'action bactériostatique du chlorure de benzalkonium.

Ces observations suggèrent la possibilité d'utiliser cet ammonium quaternaire pour éliminer des spéciments destinés à la culture du bacille tuberculeux, les germes non acido-résistants et les mycobactéries saprophytes "contaminants." Elles indiquent également que les solutions aqueuses de chlorure de benzalconium ne conviennent pas à la désinfection des matières susceptibles de renfermer le bacille de Koch.

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HYPHAE OF COCCIDIOIDES IMMITIS IN TISSUES OF THE HUMAN HOST[1]

THOMAS F. PUCKETT

(Received for publication December 21, 1953)

INTRODUCTION

It is generally accepted that human pulmonary coccidioidomycosis is acquired by inhaling the vegetative forms (chlamydospores and arthrospores) of *Coccidioides immitis*. It is also generally accepted that the parasitic forms (spherules) are not infective under normal conditions and are the usual forms found in the tissues of the host (1, 2).

The appearance of hyphae of *Coccidioides immitis* in tissues of the human host received only brief mention in the literature. Forbus and Besterbreurtje noted hyphae in only one of their ninety-five cases (3) and stated that this phenomenon had not been described previously. Mahon (4) observed hyphae in a cavitary lesion from a patient in this hospital in 1945 and subsequently collected nine other cases. Barnes (5) referred to one of Mahon's cases. Two of Mahon's cases and one case from another source were mentioned briefly by Smith, Beard, and Saito (6). Three cases from the same group were again mentioned by Greer, Forsee, and Mahon (7). Weisel and Owen (8) found hyphae in a direct smear from a resected cavitary lesion.

It is generally believed, therefore, that the presence of hyphae of *Coccidioides immitis* in tissues of the human host is an exceedingly rare finding. It is obvious that, since the vegetative forms of the organism are infective, their presence in the human host might permit human to human transmission of coccidioidomycosis. Accordingly, the present study was undertaken to determine the frequency with which mycelial structures could be found in resected focalized lesions.

MATERIAL AND METHODS

During the eight-year period between September 1, 1945, and August 31, 1953, 64 specimens of pulmonary tissue containing *C. immitis* have been examined in this laboratory. Material from all cases was available for the study. Thirty-four specimens contained cavities. Thirty specimens contained either solitary granulomatous foci (coccidioidomas) or large granulomas with smaller nodose satellite lesions. All specimens had been examined, lesions opened and the specimens placed in fixative within approximately two to three hours following surgical resection. It is not likely, therefore, that growth occurred after the surgical removal of the tissue. The disease process had been considered clinically focalized and stabilized in all cases prior to surgery. No cases of disseminated coccidioidomycosis were included in the study.

[1] From the Laboratory Service, Fitzsimons Army Hospital, Denver, Colorado.

TABLE 1

RESULTS OF CULTURES FOR C. IMMITIS FROM FRESH SPECIMENS OF RESECTED PULMONARY LESIONS

	CAVITARY LESIONS	GRANULOMATOUS LESIONS
Cases	28	17
Cultures positive	26	9
Percentage positive	93	53

TABLE 2

HYPHAE OF C. IMMITIS IN HUMAN PULMONARY TISSUE

	CAVITARY LESIONS	GRANULOMATOUS LESIONS
Total studied	34	30
Hyphae present	25	9
Percentage with hyphae	73	30

If hyphae were not found in routine sections taken from the specimen, the entire lesion was sectioned by the multiple block technique and studied. This procedure was not always practicable with the multiple granulomatous lesions, although a generous number of blocks were studied. Hyphae can be seen with difficulty in hematoxylin and eosin stained sections, but identification is much more readily made and detail rendered more clearly by the use of the periodic-acid-Schiff stain. All cases in this series were studied by the use of the latter procedure (9).

The results of culturing contents of cavities and portions of the soft material from the granulomas are shown in table 1.

A variety of standard media was used. During the past three years brain heart infusion medium with streptomycin and penicillin added was used almost exclusively for the initial culture. Duplicate plates were usually prepared, one incubated at room temperature and one at 37°C. In most instances colonies appeared in one to five days, and, in general, growth was more rapid on the plates incubated at 37°C. Cultures were held for six weeks before being discarded as negative. Cultures which were grossly typical and grossly pure were held until thorough microscopic study of the tissue was completed. If spherules with endosporulation were found in tissue sections, the cultures were reported as positive. If organisms could not be identified in tissue sections, material from broth subcultures was injected intraperitoneally into mice. The animals were sacrificed after six days and sections from their viscera were studied for spherules. This procedure was necessary in but 2 cases, one cavity and one granuloma. Re-examination of the cavitary lesion disclosed hyphae within the wall. Re-examination of the entire granuloma revealed no organisms. Typical spherules with endosporulation were present in the tissues of animals inoculated with cultures from both cases. It is assumed that organisms in the granulomatous lesion were confined to the soft central area which was removed for culture.

RESULTS

Hyphae were found in 34 specimens (55 per cent) of the lesions studied. The occurrences in the two types of lesions are shown in table 2.

The type of mycelial structures seen varied from simple branching septate hyphae (figure 1), which were the predominant forms in the solid lesions but were present in all cases, to tangled masses of hyphae and forms which appeared

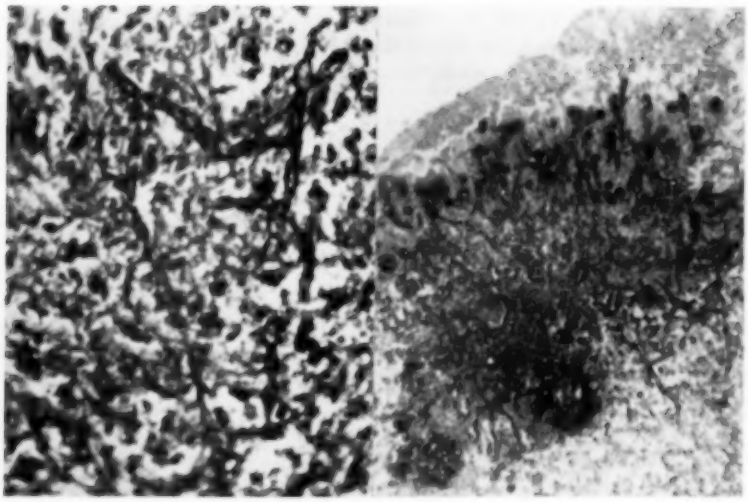

FIG. 1. (Left) Branching septate hyphae in granulomatous lesion. (Periodic-acid-Schiff stain, ×800)

FIG. 2. (Right) Growth in cavity wall; note thick-walled structures. (Periodic-acid-Schiff stain, ×350)

to contain spores. The latter forms were more predominant in the walls of the cavities (figures 2 and 3) and, although segmentation was marked and chromatin clumped, the morphology was not that of true arthrospores.

Intermediate between these types were collections of hyphae in close association with yeastlike forms (figure 4). The latter structures are believed to be endospores which, although liberated from spherules, have failed to develop further, giving rise to hyphae instead. Other round forms may be present because of the plane in which hyphae were sectioned.

Collections of fragmented spherules, as well as structures interpreted as endospores, were in close association with mycelial growth (figure 5) in several instances. The latter association was most frequently seen in the solid lesions. In figure 2 it is shown that the outermost structures have thick walls and, although endosporulation was not observed, the structures are similar to the "culture spherules" of Baker and Mrak (10). In figure 6 is shown an intercalary "culture spherule," also observed by the latter writers.

A careful search was made for evidence of endosporulation. Although chromatin was frequently separated into coarse clumps (figure 6) and there was occasionally a suggestion of segmentation within the thick-walled structures, the accepted pattern of endosporulation was not seen.

One rather striking observation was made during the study of the cavitary lesions. It was noted that those few cavities containing typical spherules of *C. immitis* within the necrotic portions of their walls or in the cellular debris within

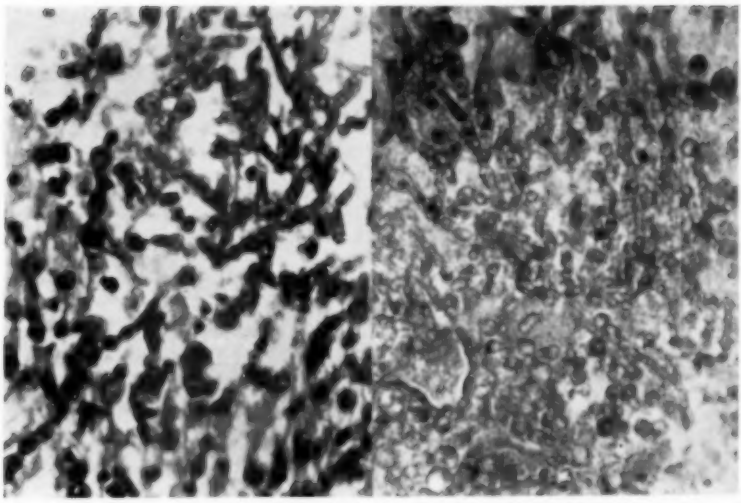

FIG. 3. (Left) Spore containing forms; note clumping of chromatin; cavity. (Periodic-acid-Schiff stain, ×800)

FIG. 4. (Right) Hyphae associated with yeastlike forms; cavity. (Periodic-acid-Schiff stain, ×800)

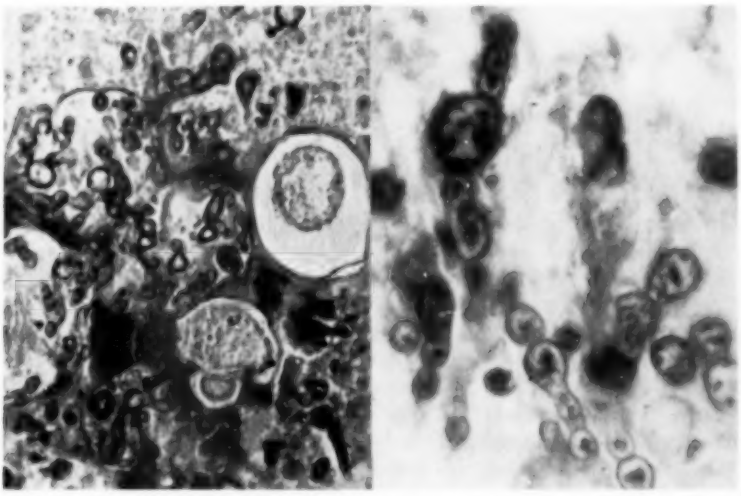

FIG. 5. (Left) Hyphae associated with fragmented spherules; granuloma. (Periodic-acid-Schiff stain, ×800)

FIG. 6. (Right) Intercalary "culture spherule"; cavity. (Periodic-acid-Schiff stain, ×1,650)

TABLE 3

RESULTS OF INTRADERMAL COCCIDIOIDIN TESTS

	CAVITARY LESIONS (TOTAL OF 28)				GRANULOMATOUS LESIONS (TOTAL OF 24)			
	C+	Per Cent	C−	Per Cent	C+	Per Cent	C−	Per Cent
With hyphae	16	76.1	5	23.9	5	55.5	4	44.5
Without hyphae	6	85.5	1	14.5	9	60.0	6	40.0
Total	22	78.5	6	11.5	14	58.2	10	41.5

C+, positive reaction to coccidioidin; C−, negative reaction to coccidioidin.

the cavity did not contain hyphae, while those cavities containing rare or no typical spherules usually contained hyphae. In the latter cases, spherules were present in granulomatous foci in the lung parenchyma adjacent to the cavity or in similar foci in the hilar lymph nodes. Forbus and Besterbreurtje (3) reported similar findings in their case. There was no similar correlation between the appearance of hyphae and absence of spherules in the solid lesions, although hyphae were frequently seen in association with distorted or partially degenerated spherules (figure 5).

Zimmerman (11) had independently noted the correlation between absence of organisms and presence of hyphae in cavitary lesions. He suspected an altered host-parasite relationship which might result in further correlation between those cases in which hyphae were found and those patients who did not react to intradermal coccidioidin. Of the 64 cases studied, 52 patients had received intradermal coccidioidin and 36 (69.3 per cent) gave a positive reaction. The reactions in both cavitary and granulomatous lesions are shown in table 3. There was a significant difference between the percentage of positive reactors in the cavitary group and those in the granulomatous group. There was little difference in percentage between the positive reactors in whom hyphae were found and those in whom hyphae were not found.

DISCUSSION

Ophüls (12) demonstrated hyphae growing from spherules in hanging drop preparations and noted the concomitant disappearance of vegetative forms and the appearance of spherules in the contents of an abscess in the ear of a rabbit. Wolbach (13) demonstrated similar transformations, and both papers are well illustrated. The development of spherules from hyphae has since been observed by others in cultures (10, 14, 15). The results of the present study indicate that hyphae can arise in the same manner in focalized lesions in the human host.

Baker and Mrak (10) believed that there was a relation between the age and dryness of their cultures and the formation of "culture spherules." It may be that there is a similar correlation for the appearance of hyphae in tissues, for all of the lesions in the present series were considered stable prior to removal. In none of the cases containing hyphae was there evidence of activity as mani-

fest by liberation of endospores and acute inflammatory reaction. Further, resorption of fluid from some of the solid lesions resulted in portions of the lesions being quite dry and friable. It is probable that there is some correlation between the age of a lesion and a negative reaction to coccidioidin. It is also probable that the older lesions are more likely to contain hyphae. The duration of disease in the case reported by Forbus and Besterbreurtje was nine years (3). It was not possible to determine the onset of the disease with reasonable accuracy in a sufficient number of cases in the present series to attempt correlation of the results with the age of the lesion.

The finding of either spherules or mycelial growth, but seldom both, in the walls of the cavities brought out the obvious possibility of the mycelial growth being that of a secondary invader of a saprophytic type. Cultures from 5 cases in which no spherules were seen within the walls or in smears of the exudate from the cavity were inoculated intraperitoneally into mice. The viscera of all animals contained typical spherules with endosporulation when sacrificed six days after inoculation. While this does not prove the absence of another fungus, it does prove the presence of *C. immitis* in the cavities even though no spherules could be identified in sections.

The probability of a saprophyte's being present is further lessened by the fact that the morphology of the mycelial growth was similar in all cases. Moreover, all cultures were grossly pure cultures and, while *C. immitis* grows very rapidly, most saprophytic fungi also grow rapidly. It is not believed likely that heavy inocula such as were used would result in complete overgrowth by the pathogen and failure to recognize a saprophyte.

It must be admitted that each lesion with mycelial elements provides a theoretical hazard for human to human transmission. A critical appraisal of the results of the present study and of the experiences of others indicates that this "theoretical hazard" is of an extremely low order. The present writer doubts that the stabilized solid lesions (coccidioidomas) in this series would ever "break down" and become potential sources of infective material.

It is more difficult to evaluate the possibilities of the cavitary lesions. No patent bronchi were described during gross examination of the cavities which were later found to contain mycelia. It is probable that some cavities communicated with the bronchial tree, however, for hyphae were present in the lumen of one small bronchus and there was partial epithelization of the cavity wall in numerous instances. Frankly patent bronchocavity junctions were uncommon but were seen. It seems probable then that expectoration of infective mycelial elements may occur occasionally. The writer knows of no instance of coccidioidomycosis resulting from "contact" infection. It is believed that the observation of mycelial growth in 55 per cent of the cases in this series resulted from careful study of the entire resected lesions by special staining techniques rather than that their presence in this series was unique. It follows, therefore, that, if hyphae are expectorated, "contacts" of patients with coccidioidomycosis have not been harmed by them in the past. Smith and his associates (6) pointed out that cases which they have observed indicated that ordinary hygienic precaution sufficed

to prevent "contagion." This concept was further supported by the studies of Bass and his associates (16) on long-term contacts of persons with coccidioidomycosis.

SUMMARY

Mycelial growth of *Coccidioides immitis* may develop in focalized and stabilized lesions in the human host in the same manner that it develops in cultures.

In the present series, hyphae were more commonly found in cavities (73 per cent) than in granulomatous lesions (30 per cent).

Mycelial growth and typical spherules are seldom present in the wall of the same cavity.

There is no relationship between mycelial growth and a negative reaction to intradermal coccidioidin.

There is no evidence that the presence of hyphae of *C. immitis* in the human host creates other than a theoretical hazard for human to human transmission of coccidioidomycosis.

SUMARIO

Hifas del Coccidioides Immitis en los Tejidos del Huésped Humano

En las lesiones focalizadas y estabilizadas en el huésped humano puede presentarse proliferación micélica del *Coccidioides immitis* del mismo modo que se presenta en cultivos.

En la serie actual, se encontraron hifas más frecuentemente en las cavernas (73 por ciento) que en las lesiones granulomatosas (30 por ciento).

Raramente existen colonias de micelios y típicas esférulas en la pared de la misma caverna.

No hay relación alguna entre la proliferación micélica y una intradermorreacción negativa a la coccidioidina.

No hay pruebas de que la presencia de hifas del *C. immitis* en el huésped humano cree más que riesgo teórico de transmisión interhumana de la coccidioidomicosis.

RESUME

Hyphes de Coccidioides immitis dans les tissus de l'hôte humain

Le développement de filaments mycéliens de *Coccidioides immitis* peut se produire chez l'homme dans des lésions focales stabilisées, de la même manière que dans les cultures.

Dans les séries étudiées, les hyphes ont été plus souvent rencontrés dans les excavations (73%) que dans les granulomes (30%).

Le développement mycélien et les corpuscules sphériques sont rarement présents dans la paroi d'une même excavation.

Le développement mycélien et une réaction négative à la coccidioïdine intradermique sont sans corrélation.

Il n'est pas démontré que la présence d'hyphes de *C. immitis* chez l'homme constitue plus qu'un danger théorique de transmission de la coccidioïdomycose de l'homme à l'homme.

Acknowledgment

The author wishes to thank Colonel H. W. Mahon, M.C. (U. S. A. retired) for permission to study his ten cases and include them in this paper.

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ELECTRON-MICROSCOPIC STUDY OF THE EFFECT OF STREPTOMYCIN ON THE CYTOLOGY OF TUBERCLE BACILLI[1]

R. VISHWANATHAN, K. C. GUPTA, A. PANDE, I. C. CHOPRA, AND A. J. H. DEMONTE

(Received for publication October 19, 1953)

Mycobacterium tuberculosis was one of the first organisms to be submitted to biologic studies with the aid of the electron-microscope. A summary of the information on the subject has recently been presented (1).

The present studies were not confined merely to the morphology of tubercle bacilli but were extended to the observation of changes in the cytology of the bacilli as a result of the action of certain antimicrobial drugs. The effects of streptomycin on a bovine strain of tubercle bacilli (B19-3 Kasauli) were observed.

METHOD

Solutions of streptomycin of concentrations varying from 1.0 mg. per ml. to 0.01 mg. per ml. were added to tubes containing a four-day-old culture of tubercle bacilli (concentration, 1.0 mg. per ml.) in Youmans' medium containing 0.05 per cent of Tween® 80. The tubes were incubated at 37°C., and specimens were taken after 18, 24, 96, and 168 hours for examination under the electron-microscope.

After centrifuging and washing with distilled water, a drop of bacillary suspension was placed on a collodion film mounted on a wire mesh, air dried, fixed over osmic acid vapor, and examined under the electron-microscope.

RESULTS

As may be seen in figure 1, the observations made were in confirmation of the findings of other workers concerning the structure of the tubercle bacillus.

No appreciable change in the morphology could be seen in specimens taken after eighteen hours of incubation with streptomycin. After thirty-eight hours, however, the bacilli were found considerably elongated and swollen at the center (figure 2). Bacilli examined after ninety-six hours showed swelling both at the center and at the end (figure 3). Figure 4 is also a specimen examined after ninety-six hours, showing disintegration of the cytoplasm. After seven days of treatment with streptomycin, most of the bacilli are found with almost complete disintegration of the cytoplasm. It is observed that, even after such a long interval, the cell wall remains intact (figure 5).

COMMENT

From a study of a large number of electron-micrographs, it becomes evident that the tubercle bacillus undergoes a series of changes in successive stages as a result of the action of streptomycin. At first the bacillus gets elongated, at a later

[1] From the Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, and Drug Research Laboratories, Jammu.

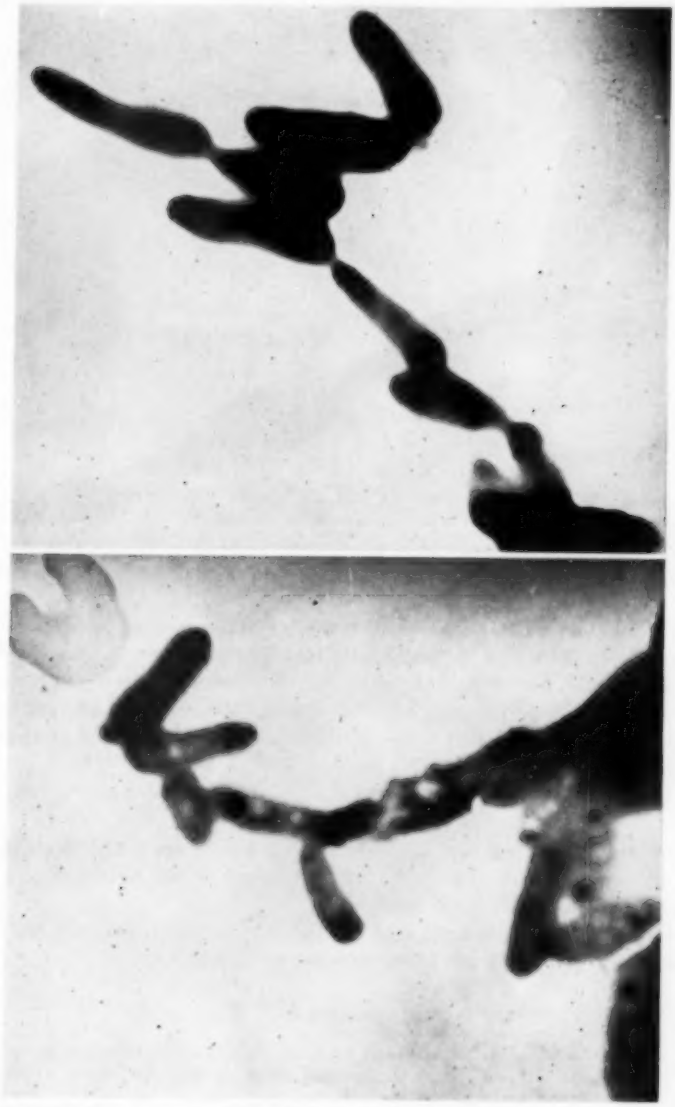

FIG. 1. (Top) From a five-day-old culture of bovine tubercle bacilli, showing the cell wall and mitochondria. (Bottom) From a five-day-old culture of bovine tubercle bacilli, showing the cell wall, mitochondria, and vacuoles. (×20,000)

FIG. 2. The bacilli examined after thirty-eight hours of treatment with 0.1 mg. of streptomycin per ml. Note the marked elongation with swelling at the center. (×20,000)

stage the middle portion of the bacillus swells up, the swelling later extending to other parts of the bacillus. At a still later stage the cytoplasm is found to disintegrate. It appears, however, that the nuclear disintegration occurs at a still later stage. In the studies so far, the cell wall appears to resist the action of the drug and continues to remain intact even after almost complete disintegration of the cytoplasm.

SUMMARY

Observations on the morphology of tubercle bacilli under the electron-microscope are reported, and the effect of streptomycin on the structure of a bovine strain of tubercle bacilli has been studied.

Under the action of the drug, such changes as elongation, swelling, and disintegration of the cytoplasm are found to occur in stages.

SUMARIO

Estudio Electrono-Microscópico del Efecto de la Estreptomicina sobre la Citología de los Bacilos Tuberculosos

Comunicanse observaciones relativas a la morfología de los bacilos tuberculosos con el microscopio electrónico, y se estudia aquí el efecto de la estreptomicina sobre la estructura de una cepa bovina del bacilo tuberculoso.

Observóse que, bajo la acción de la droga, se presentaban en etapas alteraciones tales como alargamiento, inflación y desintegración del citoplasma.

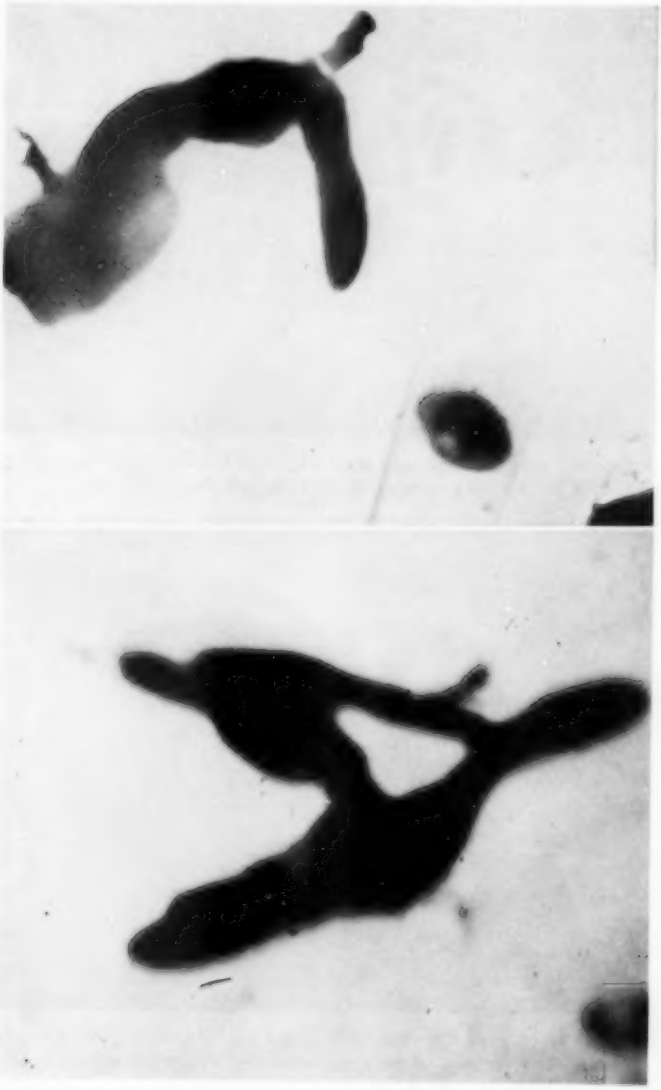

FIG. 3. The bacilli after ninety-six hours of treatment with 0.1 mg. of streptomycin per ml. Swelling both at the center and at the ends of the bacilli is well marked. (×20,000)

FIG. 4. Another specimen examined after ninety-six hours of treatment with 0.1 mg. of streptomycin per ml., showing disintegration of the cytoplasm.

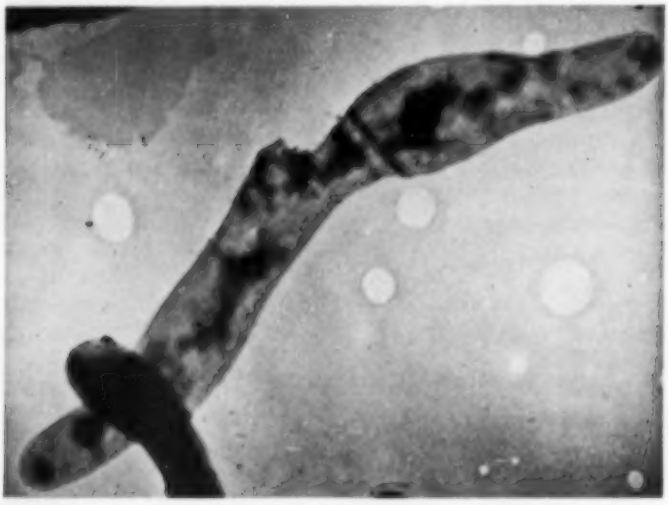

FIG. 5. After seven days of treatment with 0.1 mg. of streptomycin per ml., showing almost complete disintegration of the cytoplasm. One or two mitochondria are still seen intact. The cell wall is also seen intact.

RESUME

Etude au microscope électronique de l'action de la streptomycine sur la cytologie des bacilles tuberculeux

Des observations sur la morphologie du bacille tuberculeux à l'aide du microscope électronique sont rapportées, et l'action de la streptomycine sur la structure de bacilles tuberculeux d'une souche bovine est étudiée.

Il a été observé que, sous l'action de la drogue, des changements cytologiques tels que l'élongation, le gonflement et la désintégration du cytoplasme survenaient par stades.

Acknowledgments

The writers wish to express their grateful thanks to Col. R. N. Chopra, Director, Drug Research Laboratories, Jammu, for his helpful suggestions, and to Professor K. S. Krishman, Director, National Physical Laboratory, for his kind permission to conduct the electron-microscopic studies in that laboratory.

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THE "SEROGRAM" IN PULMONARY TUBERCULOSIS. ELECTROPHORETIC SERUM PROTEIN FRACTION CHANGES DURING ISONIAZID THERAPY[1, 2]

A Preliminary Report

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INTRODUCTION

In a previous publication (1) the writers reported on the electrophoretic and chemical serum protein fractions in fifty cases of pulmonary tuberculosis. It was observed that, in this form of tuberculous infection, there is a marked decrease in the electrophoretic serum albumin with concomitant increases in the alpha-2, beta, and gamma globulins paralleling the increasing severity of the disease. The most significant change was noted in the gamma globulin fraction, as determined either electrophoretically or by means of the quantitative protein flocculation-ninhydrin method (2, 3, 4), as seen in figure 1. These findings with respect to the gamma globulin changes have been confirmed by other investigators (5, 6, 7).

Serial studies of the electrophoretic serum proteins of fifteen selected cases of pulmonary tuberculosis were carried out during a one-year period. It was noted that the serum protein values tended to return to normal with improvement in the clinical condition of the patient. Conversely, deterioration of the patient's clinical status resulted mainly in an increase in the gamma globulin fraction accompanied by a decline in the albumin and, therefore, a significant decrease in the electrophoretic albumin-globulin ratio.

These biochemical findings seem to offer a useful tool for the objective evaluation of the clinical course of patients undergoing treatment with various therapeutic agents, such as streptomycin plus para-aminosalicylic acid (PAS) (8, 9) or with various isonicotinic acid derivatives (9). Since the routine evaluation of the electrophoretic data in following the tuberculous process of a patient is both time-consuming and difficult, a simple pictorial method has been proposed by Kanagami (10). This graphic method of representing the electrophoretic data has been termed a "serogram" and has been employed by the present writers to demonstrate the protein changes in a series of patients before and after treatment with various therapeutic agents.

[1] From the Division of Laboratories, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, New York.

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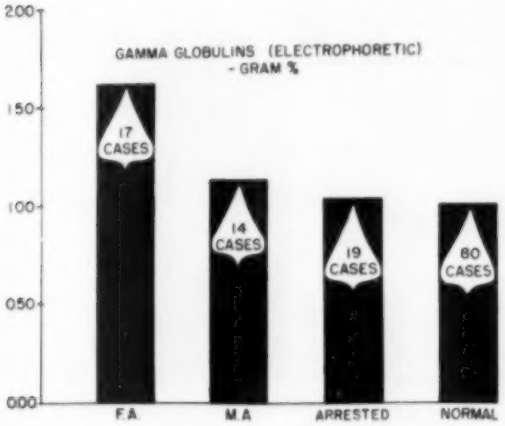

FIG. 1. Mean gamma globulin levels in various stages of pulmonary tuberculosis (50 cases), as compared with mean value of 80 normal subjects.

MATERIALS AND METHODS

The study was carried out on 10 patients with either moderately or far advanced pulmonary tuberculosis classified according to the standards of the National Tuberculosis Association (11). Of these, 9 patients received doses of 250 mg. of isoniazid daily during the course of the study without additional medication. The tenth patient was treated in the routine manner with streptomycin plus PAS (8). On each of these 10 patients, electrophoretic protein studies were performed, as previously described (1), prior to the initiation of treatment. Subsequently, serial electrophoretic protein determinations were carried out two to seven times during a four- to eight-month period. The results thus obtained were compared with the clinical and roentgenographic findings in each case.

Preparation of serograms: The method of preparing the "serogram" is shown in figure 2. The median line of the graph represents the mean value of each electrophoretic protein component. Each parallel line on either side of the center represents either a positive (right) or negative (left) standard deviation of the indicated component from its mean value. The cross-hatched area represents twice the standard deviation from the mean normal value for each electrophoretic component. As the normal serum electrophoretic values in this laboratory check closely with those obtained by Reiner, Fenichel, and Stern (12), who employed a similar instrument in their studies, their series of eighty normal persons was used to prepare the "ideal serogram" shown in figure 2.

Normal subjects: A perfectly straight line would represent the "ideal" normal electrophoretic pattern. Since this is rarely observed in practice, figure 3 demonstrates typical patterns obtained for 9 normal adults.

Serograms of patients with pulmonary tuberculosis: Serograms were prepared from the electrophoretic data obtained in the previous study (1) of fifty patients with pulmonary tuberculosis classified according to the severity of the disease (11). The serograms representative of the average values for each category of disease—*far advanced*, *moderately advanced*, and *arrested*—are shown in figure 4, as compared with the average of a series of

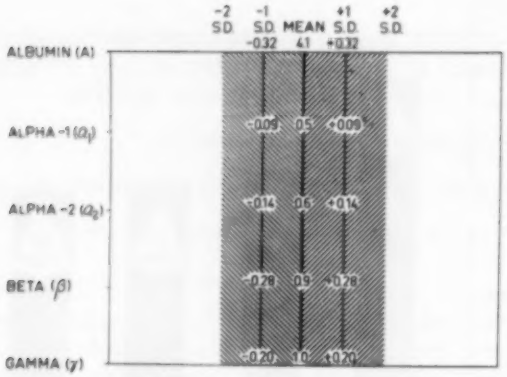

FIG. 2. The "ideal" serogram compiled from the mean electrophoretic protein fractions of 80 normal subjects and the standard deviations for each component. The cross-hatched area represents twice the standard deviation.

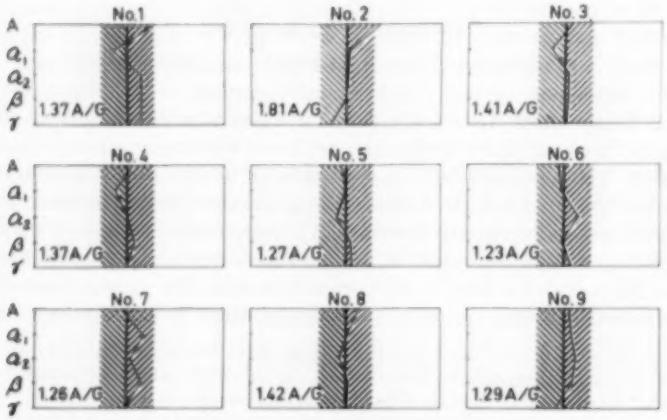

FIG. 3. Typical serograms of 9 normal subjects

twelve normal subjects. This small number of control sera were run under the same experimental conditions as were the sera of the tuberculous patients and approximate in number the specimens run for each disease category shown in figure 4. All fifty patients in this series were under standard treatment with streptomycin and PAS.

Serograms applied to serial studies of individual patients: In the attempt to follow objectively the effect of treatment with isoniazid, serograms were prepared from the electrophoretic data obtained in the serial studies of 9 patients with moderately and far advanced pulmonary tuberculosis. One additional patient treated with the standard regimen of streptomycin and PAS was also followed serially as a control with the aid of the serograms.

RESULTS

The serograms of 9 normal subjects (figure 3) were obtained from members of the laboratory staff of both sexes who were known to be in good health. It can be

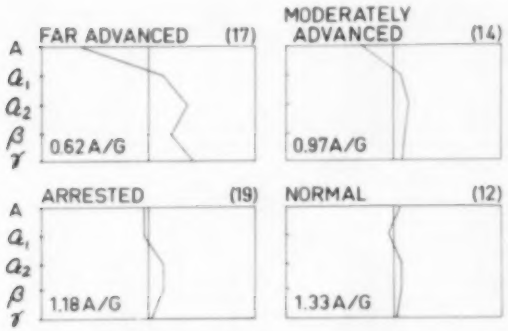

FIG. 4. Serograms representing the mean electrophoretic values of various categories of pulmonary tuberculosis as compared with the mean normal values. The figures in brackets represent the number of cases in each category.

seen that in practically no instance did the protein values obtained extend beyond the cross-hatched area, i.e., twice the standard deviation. As expected, although none of the normal subjects showed an "ideal" normal pattern, i.e., a straight line, their average would tend to approximate such a straight line (figure 4).

The serograms shown in figure 4 demonstrate the characteristic protein pattern for each category of pulmonary tuberculosis studied. It can be noted that, with the improvement in the disease process as determined clinically and roentgenographically, the serograms begin to approach normal values or approximate a straight line. However, the arrested cases still show a slight elevation of their alpha-2 and beta globulin fractions.

The serial serograms of a patient with moderately advanced pulmonary tuberculosis undergoing treatment with streptomycin and PAS are shown in figure 5. This patient showed some evidence of clinical improvement during the observation period of four and one-half months. It can be seen that this improvement in the clinical condition is reflected in the changes noted in the serograms, namely, an increase in the albumin fraction with some lowering of the gamma globulin values as compared with the initial level. This shift of albumin to the right accompanied by a shift of the gamma globulin values to the left expresses objectively the improvement in the clinical condition of the patient. The beta globulin component, which was slightly elevated above normal at the start of this study, declined temporarily but returned to approximately its original value at the end of the observation period.

Of the 9 subjects treated with isoniazid, 3 showed definite improvement during the observation period, 2 exhibited a temporary improvement followed by decline, 3 patients demonstrated stabilization of their tuberculous condition, whereas one declined rapidly and died. The improvement was evident clinically by decreased expectoration, with more frequent sputum examinations which were negative for tubercle bacilli and improvement of the general well-being with gain of appetite and weight as well as decline of temperature. Roentgenographic examination of

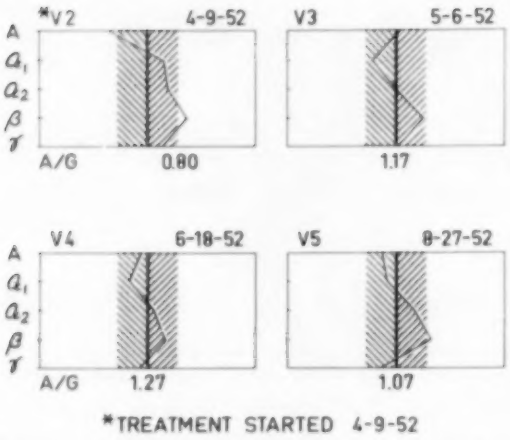

FIG. 5. Serial serograms of a patient with moderately advanced pulmonary tuberculosis undergoing treatment with streptomycin and PAS.

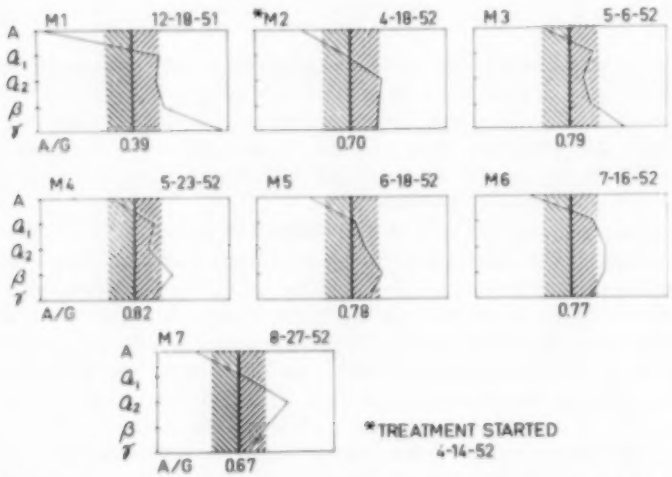

FIG. 6. Serial serograms of a representative isoniazid-treated patient who improved considerably over the initial status.

the lungs showed an arrest of the active tuberculous process and, in some instances, a considerable decrease in size and encapsulation of the cavities.

Serial serograms of a representative patient who improved considerably over his initial status during an eight-month period are shown in figure 6. The serograms show a marked increase in the albumin fraction (and the albumin-globulin ratio) with a simultaneous decline of the gamma globulins, thus reflecting the

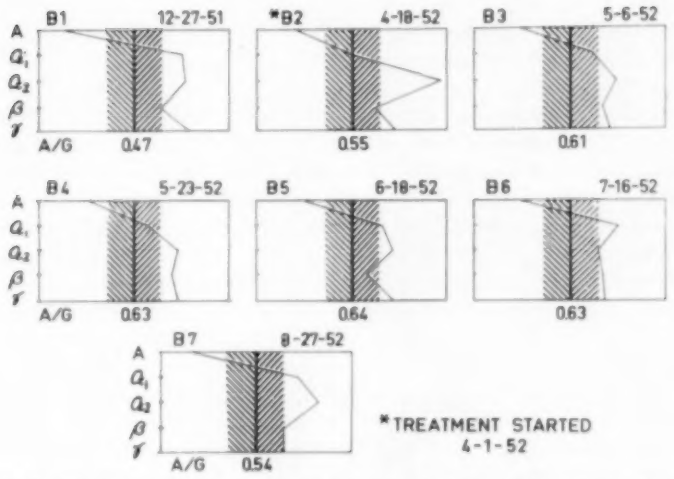

FIG. 7. Serial serograms of a representative isoniazid-treated patient who showed temporary clinical improvement.

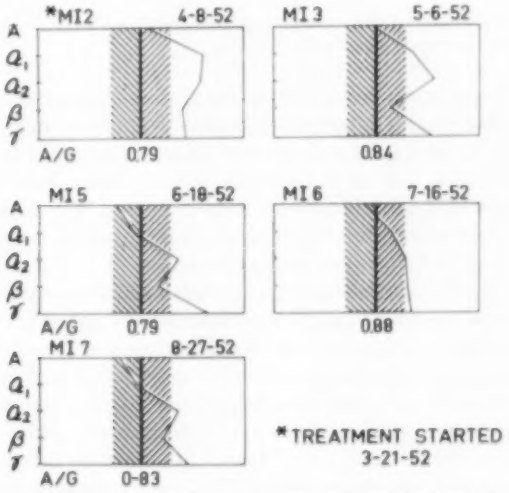

FIG. 8. Serial serograms of a representative isoniazid-treated patient who showed no clinical change during therapy.

clinical improvement of the patient. It can be noted that the alpha-2 component after a temporary decline to normal values shows the greatest increase above normal of all the protein fractions. The protein changes of the other 2 patients in this group showed a similar trend toward a normal electrophoretic pattern

The serograms shown in figure 7 are representative of the 2 patients who

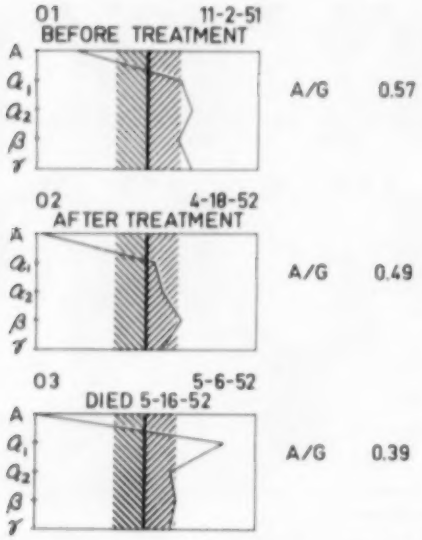

FIG. 9. Serial serograms of an isoniazid-treated patient with far advanced pulmonary tuberculosis who deteriorated rapidly until death.

showed a temporary improvement after the initiation of isoniazid treatment, which was followed by a deterioration to a clinical status similar to their initial condition. It can be seen in this figure that the initially low albumin (and the albumin-globulin ratio) tended to approach normal values, only to decline subsequently. The initially high gamma globulin decreased only slightly, while the alpha-2 fraction remained elevated during the observation period of approximately four and one-half months during treatment.

The 3 patients with stabilized pulmonary tuberculosis showed minor changes in the albumin or in the globulin fractions. This is exemplified by the serial serograms of the case shown in figure 8. The laboratory data were borne out clinically in one case, but in the other 2 patients some weight gain and improvement of appetite were recorded although otherwise no marked changes were noted.

The serograms shown in figure 9 are from a patient with pulmonary tuberculosis who was in a serious condition to begin with and who deteriorated rapidly until death. It can be noted that there is a decrease in the albumin fraction with a marked increase in the alpha-1 just prior to death with a consecutive decline of the electrophoretic albumin-globulin ratio.

DISCUSSION

From the data presented above it appears that the electrophoretic protein changes, as illustrated in the serial serograms for the isoniazid-treated patients, offer a good objective means of following the clinical progress of the patients.

It should be stressed that no definite conclusions can be drawn from the study

of such a small series of patients undergoing isoniazid treatment. However, it can be noted that the patients selected for such treatment were those who had previously undergone extensive therapy with streptomycin and PAS with little or no benefit. The improvement shown in the 3 patients discussed above is in keeping with recent reports dealing with short-term comparative clinical studies of treatment with streptomycin and PAS, as compared with isoniazid alone. It has been stated in the literature that there is more roentgenographic and clinical improvement in patients treated with isoniazid alone than in those treated with streptomycin and PAS, although the difference is not great (13, 14, 15). No data of electrophoretic protein changes were obtained in studies on the combined use of these therapeutic agents, which were reported to be more effective than either one alone (16).

The electrophoretic method is at present a time-consuming and expensive procedure for evaluating the serum protein fractions in a disease process such as pulmonary tuberculosis. The increasing use of the cheaper paper electrophoretic technique may offer a practical and efficient means for determining protein components in this disease (17, 18). The clinical data thus obtained can also be conveniently expressed in the form of "serograms." Such studies are presently in progress at this laboratory.

SUMMARY

A graphic method of presenting electrophoretic data termed the "serogram" is presented. The average mean normal value for each protein fraction is represented by a straight line. Significant variations from the normal, such as occur in pulmonary tuberculosis, are readily visualized by the changes of the "serogram" pattern.

Electrophoretic data (as serograms) were used to follow serially for a four- to 8-month period 9 patients with active pulmonary tuberculosis treated with isioniazid and one patient treated with streptomycin and PAS.

Of the isoniazid-treated patients, 3 showed definite clinical and roentgenographic improvement, 2 exhibited temporary improvement followed by decline, 3 remained unchanged, and one patient declined rapidly and died.

The changes in the "serograms" during treatment appeared to follow the clinical status of the patient and provide an objective method for evaluating the course of the tuberculous process.

SUMARIO

El "Serograma" en la Tuberculosis Pulmonar. Alteraciones de la Fracción Electroforética de la Seroalbúmina Durante la Terapéutica con Rimifón: Informe Preliminar

Ofrécese un método gráfico denominado "serograma" para presentar datos electroforéticos. El valor normal medio para cada fracción de proteína está representado por una línea recta. Por medio de las modificaciones del patrón del "serograma" se visualizan fácilmente las desviaciones importantes de lo normal, como las que se observan en la tuberculosis pulmonar.

Usáronse datos electroforéticos (en forma de serogramas) para observar consecutivamente durante un período de cuatro a ocho meses a 9 enfermos con tuberculosis pulmonar activa tratados con isoniacida y a un enfermo tratado con estreptomicina más PAS.

De los enfermos tratados con isoniacida, 3 revelaron mejoría clínica y radiográfica bien definida, 2 manifestaron mejoría temporal seguida de empeoramiento, 3 permanecieron inalterados y un enfermo empeoró rápidamente y falleció.

Las alteraciones de los "serogramas" durante el tratamiento parecieron conformarse al estado clínico del enfermo y suministrar un método objetivo para justipreciar la evolución del proceso tuberculoso.

RESUME

Le "Sérogramme" dans la tuberculose pulmonaire. Variations électrophorétiques de la fraction protéique du sérum au cours du traitement par le Rimifon: Un rapport préliminaire

Présentation d'une méthode graphique concernant les données électrophorétique sous la dénomination de "sérogramme". La moyenne des valeurs moyennes normales pour chaque fraction protéique est représentée par une ligne droite. Les variations significatives de la normale, telles qu'elles se manifestent dans la tuberculose pulmonaire, sont aisément visualisées par les modifications du tracé du "sérogramme".

Les données électrophorétiques (sous forme de sérogramme) ont été utilisées pour l'observation en série, pendant une période de quatre à huit mois, de 9 malades atteints de tuberculose pulmonaire en activité qui étaient traités par l'isoniazide et de 1 malade traité par l'association streptomycine-PAS.

Parmi les cas traités par l'isoniazide, on a observé: une amélioration clinique et radiologique chez trois d'entre eux, une amélioration temporaire suivie de rechute chez deux, aucune modification chez trois, plus un cas avec aggravation rapide et décès. Les modifications des "sérogrammes" au cours du traitement apparaissent comparables à l'état clinique du malade et elles représentent un moyen d'évaluation objective du processus tuberculeux.

Acknowledgments

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SERUM PROTEIN STUDIES IN EXPERIMENTAL TUBERCULOSIS OF THE GUINEA PIG[1, 2]

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INTRODUCTION

The effects of pulmonary tuberculosis on the proteins in human serum have been the subject of a number of investigations (1). Little information, however, is available with regard to the influence of the experimental infection on the serum proteins of the guinea pig. Inasmuch as the guinea pig is widely employed as an experimental animal in evaluating the efficacy of antimicrobial drugs on the disease process, it seemed advisable to study the effects of the experimental infection on the serum proteins of this animal species. An investigation of the post-treatment effects of corticotropin and of cortisone on experimental tuberculosis (2) presented an opportunity for such a study. The present communication reports on the observations made with respect to the total serum protein, serum albumin, serum globulin, and the albumin-globulin ratio.

MATERIALS AND METHODS

Serum: One hundred and seventy-five male and female guinea pigs with an average weight of 645 gm. were observed to be nonreactors to the intracutaneous injection of 0.1 ml. of a 5.0 per cent solution of Old Tuberculin. The animals were inoculated subcutaneously in the inguinal region with 1.0 ml. of Dubos medium containing approximately 60,000 organisms of the B88 strain of *Mycobacterium tuberculosis* var. *hominis*.[3] The animals were housed 5 per cage in an air-conditioned room and were maintained on a diet of rabbit pellets supplemented with greens, and tap water, ad libitum. Four weeks after inoculation, treatment was initiated as outlined in table 1 and was continued for twenty-one days. The guinea pigs were allowed to survive sixty-eight days after the termination of treatment and were then sacrificed by exsanguination from the heart and air embolism. The serum specimens were separated and stored at −20°C. until the determinations could be conducted. Blood was also obtained by cardiac puncture from 21 normal animals and treated in a similar manner.

Chemical analyses: Total serum protein was determined by the biuret method of Weichselbaum (3). Serum albumin was estimated by the same procedure after the separation of the serum globulin by the technique of Kingsley (4). Serum globulin was determined by subtracting the value for serum albumin from the total serum protein value.

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[1] From the Department of Infectious Diseases, School of Medicine, The University of California, Los Angeles, California; and the Veterans Administration Hospital, San Fernando, California.

[2] This investigation was supported in part by research grants from the Veterans Administration; and the National Microbiological Institute, of the National Institutes of Health, Public Health Service.

[3] This strain was originally isolated from a patient with active pulmonary tuberculosis by Dr. C. Richard Smith, Barlow Sanatorium, Los Angeles, California.

TABLE 1

SERUM PROTEIN CONCENTRATIONS IN EXPERIMENTAL TUBERCULOSIS

GROUP	NUMBER OF ANIMALS*	TREATMENT	AVERAGE INDEX OF TUBERCULOSIS†	TOTAL SERUM PROTEIN		SERUM ALBUMIN		SERUM GLOBULIN		ALBUMIN-GLOBULIN RATIO	
				Mean‡	P	Mean‡	P	Mean‡	P	Mean‡	P
				gm. per 100 ml.		*gm. per 100 ml.*		*gm. per 100 ml.*			
A, Normal	21/21	None	0.0	5.4 ± 0.08		3.0 ± 0.07		2.4 ± 0.05		1.25 ± 0.05	
B, Infected	15/20	None	9.3	5.9 ± 0.12	0.001	2.8 ± 0.08	0.1	3.1 ± 0.15	0.001	0.90 ± 0.07	0.001
C1, Infected	16/20	0.1 mg. of corticotropin per day	9.8	6.6 ± 0.09	0.001	3.0 ± 0.07	—	3.6 ± 0.08	0.001	0.83 ± 0.03	0.001
C2, Infected	11/20	1.0 mg. of corticotropin per day	9.0	6.3 ± 0.12	0.001	2.8 ± 0.08	0.1	3.5 ± 0.13	0.001	0.80 ± 0.04	0.001
C3, Infected	8/20	10.0 mg. of corticotropin per day	10.1	6.5 ± 0.19	0.001	2.9 ± 0.07	0.5	3.6 ± 0.20	0.001	0.80 ± 0.06	0.001
D1, Infected	13/20	0.2 mg. of cortisone per day	8.0	6.3 ± 0.12	0.001	3.0 ± 0.09	—	3.3 ± 0.14	0.001	0.91 ± 0.06	0.001
D2, Infected	14/20	2.0 mg. of cortisone per day	9.6	6.3 ± 0.13	0.001	2.6 ± 0.04	0.001	3.7 ± 0.12	0.001	0.70 ± 0.02	0.001
D3, Infected	10/20	20.0 mg. of cortisone per day	10.7	6.1 ± 0.16	0.001	2.8 ± 0.09	0.2	3.3 ± 0.11	0.001	0.85 ± 0.03	0.001
E, Infected	6/10	10.0 mg. of beryllium chloride 3 times weekly	13.2	6.3 ± 0.28	0.001	2.7 ± 0.13	0.1	3.6 ± 0.23	0.001	0.75 ± 0.06	0.001
F1, Infected	7/10	30.0 mg. of streptomycin per day	5.7	6.2 ± 0.10	0.001	3.0 ± 0.15	—	3.2 ± 0.20	0.001	0.94 ± 0.03	<0.01
F2, Infected	9/10	30.0 mg. of streptomycin once a week	8.7	6.2 ± 0.13	0.001	2.9 ± 0.07	0.4	3.3 ± 0.12	0.001	0.88 ± 0.04	0.001

* Numerator = number of surviving animals from which sufficient blood was obtained for chemical analysis. Denominator = original number in group.

† Each organ (spleen, liver, lungs, lymph nodes) was assigned a value of 0 to 4 according to the amount of macroscopic tuberculosis. Maximum total for each animal was 16. Index of tuberculosis was the average total for each group (6).

‡ Including the standard error of the mean calculated from the relation

$$Em = [\Sigma d^2/(N(N-1))]^{1/2}$$

where "d" is the deviation from the mean and N is the number of observations.

Analysis of results: The significance of the deviations from the normal means was computed by the conventional "*t*" test (5).

RESULTS

The summaries of the treatment regimens, average indices of tuberculosis, results of chemical analyses, and statistical data are presented in table 1.

Total serum protein: Normal serum values ranged from 4.8 to 6.2 gm. per 100 ml., with a mean of 5.4 gm. per 100 ml. A significant hyperproteinemia occurred in all infected groups. The serum protein concentrations of the infected groups which received treatment were higher than the corresponding level of the infected, untreated control group of animals (*B*). The elevation was significant ($P = 0.01$) for *Groups C1* and *C3*.

Serum albumin: The average value for serum albumin in normal guinea pig serum was 3.0 gm. per 100 ml., or 55.5 per cent of the total serum protein. The latter value is in excellent agreement with the result reported by Moore (7), 54 per cent, which was obtained by electrophoretic analyses. Seven of the ten infected groups had a mean value less than the average of the normal control group (*A*). The differences were not significant with the exception of *Group D2*.

Serum globulin: The mean for the serum globulin in the 21 normal animals of *Group A* was 2.4 gm. per 100 ml. A highly significant hyperglobulinemia occurred in all infected groups. The serum globulin concentrations of the inoculated groups which received treatment were higher than the corresponding concentration for the infected, untreated control animals of *Group B*. The elevations were significant at the 1 per cent level for *Groups C1*, *C3*, and *D2*, and at the 5 per cent level for *Group C2*.

Albumin-globulin ratio: The albumin-globulin ratio determined for the normal guinea pigs was 1.25. The value is in good agreement with that reported by Moore, 1.17, based on electrophoretic data (7). A statistically significant reduction of the ratio occurred in all infected groups.

DISCUSSION

Total serum protein, serum albumin, serum globulin, and the albumin-globulin ratio are determined routinely in the clinical laboratories of tuberculosis hospitals and provide supplementary information for diagnosis and prognosis. In the present investigation, procedures comparable to the methods used in clinical laboratories have been employed to study the serum proteins of guinea pigs infected with human strain of *Mycobacterium tuberculosis*.

The pattern of the serum protein response of the guinea pig was similar to that reported for human pulmonary tuberculosis (1) in three respects: slight decrease in serum albumin, significant hyperglobulinemia, and a decreased albumin-globulin ratio.

One marked dissimilarity between the values in guinea pigs and in man was the concentration of total serum protein. Gutman (1), in a summary of clinical investigations, reported that the total serum protein tended to remain within normal limits although occasional moderate hyperproteinemia or hypoproteine-

mia was noted. In the present study, a statistically significant hyperproteinemia occurred in all infected groups, irrespective of the nature of treatment. Only 5 of the 109 infected animals developed a slight hypoproteinemia. Whether the variance is due to species or reflects a divergence between the acute, experimental infection of a susceptible animal and the chronic disease in relatively resistant man requires further investigation.

The results of the serum protein determinations paralleled to a certain extent the amount of macroscopic tuberculosis. Only *Group E*, which received beryllium chloride, and *Group F1*, treated with streptomycin, exhibited a significant difference in the average index of tuberculosis when compared with the infected, untreated animals of *Group B* (2). The nature and extent of the pulmonary lesions of the inoculated groups (2) would place them in the far advanced, active tuberculosis category as classified by clinical criteria (8).

The observation that the concentrations of total serum protein and serum globulin were significantly elevated in some of the groups administered corticotropin[4] or cortisone[5] when compared with the means of the infected, untreated control group (*B*) suggested that hormone treatment might have enhanced the degree of the response. The cause of the increased serum levels in the treated groups is apparently more complex since normal guinea pigs administered corticotropin or cortisone have been observed to have total serum protein levels within the normal range for the species (9).

SUMMARY

Total serum protein, serum albumin, serum globulin, and the albumin-globulin ratio have been determined in guinea pigs infected with the B88 strain of *Mycobacterium tuberculosis* var. *hominis* and treated with three graduated dosages of corticotropin and cortisone, two dosages of streptomycin, and beryllium chloride. Statistically significant increases were observed in the total serum protein and serum globulin, and a highly significant decrease occurred in the albumin-globulin ratio of all infected groups in comparison with the values of a normal control group.

SUMARIO

Estudios de la Proteína Sérica en la Tuberculosis Experimental del Cobayo

Determináronse la seroproteína total, la seroalbúmina, la seroglobulina y la proporción de albúmina/globulina en los sueros de cobayos infectados con la cepa B88 del *Mycobacterium tuberculosis* var. *hominis* y tratados con tres dosis graduadas de corticotropina y cortisona, dos tandas de estreptomicina y cloruro de berilio. Se observaron aumentos de importancia estadística en la seroproteína total y la seroglobulina y hubo una disminución de mucha importancia en la proporción A/G en todos los grupos infectados, comparados con las cifras en un grupo testigo normal.

[4] The corticotropin (Actrope®) used in the investigation was obtained through the courtesy of Mr. S. S. Kingman and Mr. R. C. Bruner, United Laboratories, Ltd., Pasadena, California. One milligram of Actrope® is equivalent to one U. S. P. unit.

[5] A large part of the cortisone acetate (Cortone®) employed in this study was obtained through the courtesy of Dr. J. M. Carlisle, Merck & Co., Rahway, New Jersey.

RESUME

Etude des protéines sériques dans la tuberculose expérimentale du cobaye

La protéine sérique totale, sérumalbumine et sérumglobuline et le rapport albumine/globuline ont été déterminés dans le sérum de cobayes inoculés avec la souche B88 de *Mycobacterium tuberculosis* var. *hominis* et traités par trois doses progressives de corticotrophine et de cortisone, deux doses de streptomycine et de chlorure de béryllium. Il s'est produit un accroissement statistiquement significatif de la protéine sérique totale et de la sérumglobuline et une diminution très significative du rapport A/G dans tous les groupes tuberculisés, par comparaison avec les valeurs obtenues dans le groupe témoin.

Acknowledgment

The assistance of Miss Helene E. Drusch and Mr. James N. Miller in the treatment of the animals is gratefully acknowledged.

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LABORATORY DIAGNOSTIC PROCEDURES EMPLOYED ON SPUTUM SPECIMENS FROM AMBULATORY TUBERCULOUS PATIENTS TREATED WITH ISONIAZID[1]

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INTRODUCTION

From a public health point of view, any decrease in the number of persons discharging tubercle bacilli in the sputum which breaks the chain of communicability and interrupts the spread of tuberculosis is of great value. This is even more so in the case of ambulatory tuberculous patients. It is important, therefore, to establish for public health laboratories suitable, accurate, and reproducible test procedures.

When the New York City Department of Health began a study of the clinical effect of isoniazid[2] on ambulatory patients, the opportunity presented itself to observe the effectiveness of laboratory diagnostic procedures as applied to specimens from these treated patients. Observations were made on the efficiency of the results of culture and microscopy; a semiquantitative method for reporting culture results on successive specimens; measurements of the appearance of drug-resistant strains of bacilli; and measurements of the effect of treatment on the results obtained by culture.

Specimens from 88 ambulatory patients with fibrocaseous tuberculosis were studied during an eight-month course of treatment consisting of 3 mg. of isoniazid per kg. of body weight.

OBSERVATIONS

Methods and materials for diagnosis by microscopy and culture: Sputum specimens from isoniazid-treated patients were received in the laboratory weekly during the first three months and every other week during the next five months of the study period. The sputum specimens were concentrated by the sodium hydroxide-hydrochloric acid method and approximately 0.25 ml. of each concentrate was spread on one slant of each of Löwenstein-Jensen (1) and Peizer-Scheehter (2) media. The cultured tubes were incubated at 37°C. for three weeks and examined for growth of *Mycobacterium tuberculosis*. Cultures negative for *M. tuberculosis* at this time were incubated for three more weeks and examined at weekly intervals. The culture results were reported by estimating the amount of growth. This procedure was found to be useful in following the bacteriologic progress of ambulatory patients under isoniazid treatment. The method used for the semiquantitative estimate of growth follows:

4-plus = confluent growth, no particulate colonies
3-plus = particulate colonies, too numerous to count
2-plus = 150 colonies to too numerous to count
1-plus = 50 to 150 colonies
less than 1-plus = less than 50 colonies, number of colonies reported as found

[1] From the Bureaus of Laboratories and Tuberculosis, Department of Health, New York City.

[2] Pyricidin®.

This method of estimating amount of growth was being used by this laboratory in the study of streptomycin susceptibility (3).

Smears were prepared by spreading 0.25 ml. of the concentrate on an area of 2 sq. cm. The smears were made simultaneously with cultures from each concentrated specimen. They were stained by the Ziehl-Neelsen technique and, on microscopy, at least fifty fields of each spread were examined by use of the Gaffky scale.

Results

Of 1,327 specimens examined simultaneously by microscopy and culture, 1,132 were positive for tubercle bacilli by either the culture, microscopy, or both procedures. Of these, 348 (30 per cent) of the 1,132 positive cultures were negative for acid-fast bacilli by microscopy. The culture and microscopy results are summarized in table 1. It is seen that, as the number of typical colonies of *M. tuberculosis* decreased, the number of negative microscopy results for acid-fast bacilli increased. This microscopy-culture relationship is also illustrated in figure 1, where it is seen that, when the *M. tuberculosis* colony evaluation was low, a low Gaffky count of acid-fast bacilli or a "negative" result was predominant.

From a practical public health point of view, this finding is of importance, since an ambulatory patient under treatment may become bacteriologically "negative" by microscopy, but on culture the identical specimen may continue to yield abundant growth of tubercle bacilli. As may be seen in table 2, of the 1,132 positive reports, 27 (2.4 per cent) were found to be negative for *M. tuberculosis* on culture and positive for acid-fast bacilli on microscopy. However, as may be seen in table 2, more than 50 per cent, or 18, of the 27 specimens reported negative for acid-fast bacilli gave a Gaffky I reading, which indicates that only one to four acid-fast organisms were found in fifty fields.

The amount of growth of *M. tuberculosis* on culture was evaluated as 4-plus, 3-plus, 2-plus, 1-plus, less than 1-plus, as indicated above. As fairly equal amounts of sputum concentrate were used for each culture and microscopic examination, it was possible to determine for each specimen the relationship of the number of acid-fast bacilli counted on each of fifty fields and the approximate number of

TABLE 1

RELATIONSHIP BETWEEN NEGATIVE RESULTS ON MICROSCOPY AND POSITIVE RESULTS ON CULTURE OF ALIQUOTS OF SAME SPUTUM SPECIMEN

1,327 Concentrated Sputum Specimens Examined—1,132 Positive Results

NUMBER OF SPECIMENS	CULTURE RESULT	PER CENT OF 348 NEGATIVE ON MICROSCOPY	PER CENT OF 1,132 KNOWN POSITIVE
126	<1+	36.2	11.11
142	1+	40.9	12.54
49	2+	14.0	4.32
23	3+	6.6	2.03
8	4+	2.3	0.70
Total, 348		100	30.7

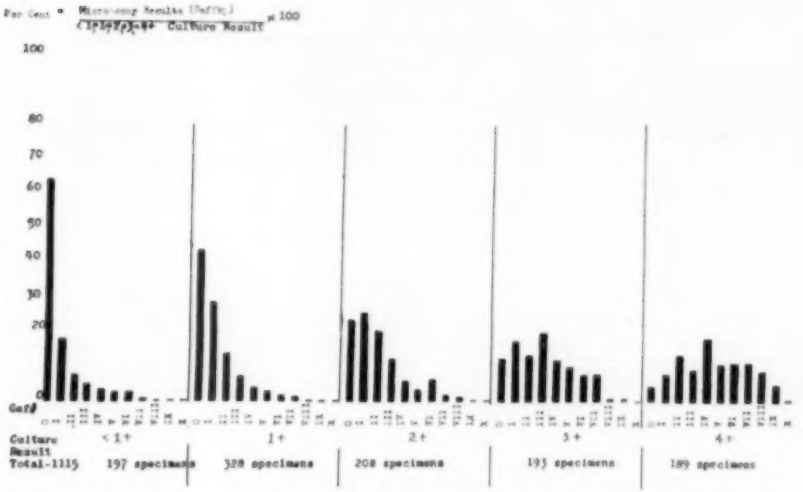

FIG. 1. Correlation of culture results with results of microscopy (Gaffky counts).

colonies produced on culture. From the results as presented in figure 2, it appears that the culture growth reported as less than 1-plus, 1-plus, 2-plus, et cetera, varied directly with the Gaffky scale. This relationship was consistent during the entire study and indicated the following relationship:

Negative microscopy: Culture growths estimated as less than 1-plus and 1-plus equaled 77 per cent of the 348 concentrated specimens which were negative on microscopy. The less than 1-plus and 1-plus culture results were sharply differentiated from the 2-plus, 3-plus, or 4-plus results.

Gaffky I and II: The less than 1-plus and 1-plus culture growths were differentiated from each other. Concentrates with Gaffky I estimates on microscopy yielded the greatest per cent of less than 1-plus colony growths.

Gaffky II: Results yielded the greatest per cent of 1-plus colony growth.

Gaffky III: The 3-plus culture growth was predominant.

Gaffky IV, V, VI, VII: The 3-plus and 4-plus culture growths were clearly differentiated from the 2-plus results.

Gaffky VIII, IX, X: Four-plus estimates of culture growth predominated, being differentiated from 3-plus.

There was a gradual ascending scale from less than 1-plus to 4-plus of *M. tuberculosis* colony evaluations which tended to run parallel with the acid-fast bacilli estimations in terms of the Gaffky scale.

As sputum was collected at regular and comparatively short intervals, it was possible to study the variability of the successive *M. tuberculosis* culture growths from specimens of each patient. The colony evaluation obtained from any one specimen of a patient was compared with that obtained from the same patient each succeeding week and the variation was noted. This comparison was made with succeeding specimens of all patients under treatment. Two groups of pa-

TABLE 2

RELATIONSHIP BETWEEN NEGATIVE CULTURE AND POSITIVE RESULTS ON MICROSCOPY

1,327 Specimens Examined

NUMBER OF SPECIMENS	MICROSCOPY GAFFKY NUMBER	PER CENT OF THE 1,132 SPECIMENS POSITIVE ON CULTURE
18	I	1.59
5	II	0.44
1	III	0.08
2	IV	0.16
0	V–VII	0.00
1	VIII	0.08
0	IX–X	0.00
Total, 27		2.35

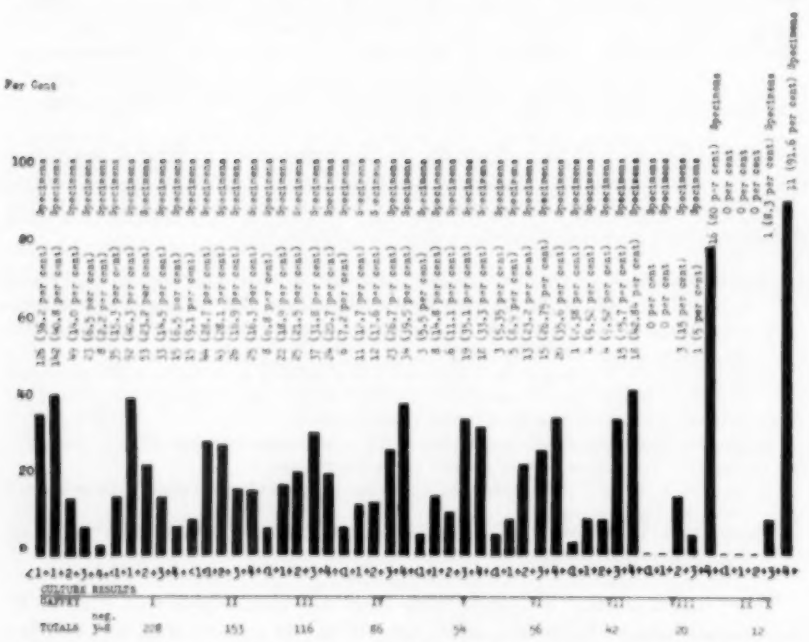

FIG. 2. Comparison of culture evaluation with Gaffky count on microscopy of 1,115 concentrated sputum specimens from patients with fibrocavernous tuberculosis.

tients were thus studied: *Group A* consisted of patients whose sputum during the period of study eventually became negative for tubercle bacilli on culture; *Group B* consisted of patients whose sputums were at first very high in acid-fast bacillary content but in whom the numbers of tubercle bacilli were reduced dur-

TABLE 3

VARIATION IN CULTURE RESULTS* OF SPECIMENS EXAMINED WEEKLY OR FORTNIGHTLY AND REPORTED AS NEGATIVE, <1+, 1+, 2+, 3+, 4+

DEGREE OF VARIATION	NUMBER OF COMPARISONS	PER CENT	
Group A (See text)			
None	39	26.0	
<1+	42	28.0	92
1+	57	38.0	
2+	11	7.2	
3+	1†	0.66	
4+	0	0.00	
Group B (See text)			
None	213	42.3	
<1+	40	7.9	86.3
1+	182	36.1	
2+	58	11.5	
3+	11‡	2.1	
4+	0	0	
Total	504	100	

* Each sputum result was compared with the following week's result of the same patient for a period of thirty weeks.

† Initial drop in number of organisms following administration of the isoniazid.

‡ Four were initial decreases following administration of the isoniazid.

ing treatment and who later reverted again to a high count as treatment continued.

In table 3 are summarized the above results, which indicate that, when any two consecutive sputum concentrates were cultured one to two weeks apart, 86 to 92 per cent of the growth observed did not vary more than 1-plus. In other words, in spite of any possible variation in the collection and transportation of the specimens to the laboratory and any technical error in the laboratory, the degree of difference, from week to week, in 86 to 92 per cent of the cultured specimens was not greater than from a 4-plus to a 3-plus reading or from a 1-plus to a 2-plus evaluation.

It would seem, therefore, that a growth variation of 1-plus, more or less, may be the expected limit when sputum of any patient is examined at weekly intervals. Any greater difference in amount of growth at any one time was usually corrected on the subsequent examination unless the change in the number of colonies of *M. tuberculosis* represented a true alteration in the bacillary content of the sputum.

Comment: The usual laboratory procedure is to report the results of microscopy or culture as either positive or negative for acid-fast or tubercle bacilli. The frequency of finding acid-fast organisms in the sputum is then taken as a measure of the patient's progress. If a patient's sputum converts from positive for acid-

fast bacilli to negative by the use of microscopy alone, there might be justification for interpreting the findings as a bacteriologic improvement. Nevertheless, the sputum may be negative for acid-fast bacilli by microscopy but still contain a large number of viable tubercle bacilli. The statement has been made (4) that 100,000 acid-fast bacilli per ml. of sputum must be present before their detection is evident by microscopy. It is apparent, therefore, that a negative result by microscopy cannot be accepted as indicating the absence of tubercle bacilli from the specimen examined (5).

On the other hand, the mere reporting of cultures as positive for *M. tuberculosis* does not distinguish between very small or very large numbers of organisms present in the sputum. This is important from a public health point of view, for it affects the spread of tuberculosis by ambulatory patients. The proposed procedure of evaluating the number of colonies of *M. tuberculosis* produced on culture from a series of specimens can be considered a rough index of the acid-fast bacillary content during treatment procedures. That this culture evaluation technique can serve as a public health tool to study the effectiveness of treatment of the ambulatory patient during various phases of his treatment schedule is indicated by a correlation found between clinical findings and the amount of colony growth of *M. tuberculosis* from consecutive series of specimens (6).

Results

Materials and methods of the drug-susceptibility test: Isoniazid was incorporated into Löwenstein-Jensen and Peizer-Scheehter media. Dilutions of the drug per milliliter of medium were 0.1 γ per ml, 0.5 γ per ml, 1 γ per ml., and 5 γ per ml. An equal amount (0.25 ml.) of the original concentrate of each sputum was inoculated on both drug-containing media. Control media containing no drug were included. The cultures were incubated at 37°C. and examined when the control reached maximum growth, which usually occurred at the end of three or four weeks of incubation. A culture was considered resistant to the highest concentration of drug per milliliter of medium on which it grew. Degree of resistance was recognized by comparison of amount of growth on the greatest concentration of isoniazid with growth on the control slants.

None of the pretreatment specimens of the 88 patients in this study showed any resistance to 0.1 γ of drug per ml. of medium, as may be seen in table 4. In this table are also summarized the resistance and culture results at the end of four weeks of treatment with isoniazid. It is of special interest that, at the end of the four-week treatment period, none of the 33 patients whose sputum was recorded as either negative or less than 1-plus growth discharged tubercle bacilli resistant to a concentration as low as 0.1 γ of isoniazid per ml. of medium. However, when only a few colonies of *M. tuberculosis* resistant to this concentration of isoniazid appeared, the culture growth usually remained 1-plus or greater.

In table 5 is shown the continued increase of isoniazid-resistant tubercle bacilli between the fourth and eighth weeks of treatment, as well as the correlation between isoniazid resistance and the *M. tuberculosis* colony count. It will be noted that, if any degree of isoniazid-resistance appeared in the bacilli discharged by a patient, such resistance not only persisted but increased during the second month of treatment with the drug. This was evident, even when the maximum growth of resistant colonies was less than 1-plus in 0.1 γ of isoniazid per ml. of

TABLE 4

COMPARISON OF DRUG RESISTANCE OF CULTURES ISOLATED FROM 88 PATIENTS BEFORE TREATMENT AND AT END OF FOUR WEEKS OF TREATMENT IN TERMS OF AMOUNT OF COLONY GROWTH OF M. TUBERCULOSIS

WEEKS OF TREATMENT	NUMBER OF PATIENTS	GROWTH* ON MEDIUM WITH ISONIAZID	GROWTH* ON MEDIUM WITHOUT ISONIAZID					
			Negative	<1+	1+	2+	3+	4+
		γ per ml.						
Pretreatment	88	0	2	8	11	16	16	35
4	74	0	13	20	14	15	8	4
	7	<1+ growth in 0.1 γ	0	0	5	2	0	0
	7	0.1 γ	0	0	3	1	3	0
			13	20	22	18	11	4

* At least 1+ growth, except where <1+ is recorded.

TABLE 5

APPEARANCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI IN 88 TREATED PATIENTS RELATED TO DURATION OF THERAPY AND TO COLONY COUNT AT THE END OF FOUR AND EIGHT WEEKS OF TREATMENT

PATIENTS WITH TUBERCLE BACILLI RESISTANT TO ISONIAZID AT END OF FOUR WEEKS OF TREATMENT		END OF EIGHT WEEKS OF TREATMENT							
				Growth* on Medium without Isoniazid					
Number of Patients	Growth* on Medium with Isoniazid	Number of Patients	Growth* on Medium with Isoniazid	Negative	<1+	1+	2+	3+	4+
	γ per ml.		γ per ml.						
74	0	37	0	19	6	12	0	0	0
		5	<1+ in 0.1	0	1	2	0	1	1
		22	0.1	0	0	12	3	4	3
		3	0.5	0	0	1	0	2	0
		6	1.0	0	0	2	1	0	3
		1	5.0	0	0	0	1	0	0
7	<1+ in 0.1	3	0.1	0	1	1	1	0	0
		0	0.5	0	0	0	0	0	0
		3	1.0	0	0	0	1	1	1
		1	5.0	0	0	1	0	0	0
7	0.1	3	0.1	0	0	0	1	2	0
		1	0.5	0	0	0	0	0	1
		2	1.0	0	0	0	0	2	0
		1	5.0	0	0	0	0	1	0
88		88		19	8	31	8	13	9

* At least 1+ growth except where <1+ is indicated.

TABLE 6

APPEARANCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI AND RELATIONSHIP OF RESISTANCE TO COLONY EVALUATION AFTER EIGHT AND TWELVE WEEKS OF TREATMENT

RESISTANCE OF TUBERCLE BACILLI CULTURES ISOLATED AFTER EIGHT WEEKS OF TREATMENT		END OF TWELVE WEEKS OF TREATMENT							
				Growth* on Medium without Isoniazid					
Number of Patients	Growth* on Medium with Isoniazid	Number of Patients	Growth* on Medium with Isoniazid	Negative	<1+	1+	2+	3+	4+
	γ per ml		*γ per ml.*						
36	0	21	0	16	5	0	0	0	0
		1	<1+ in 0.1	0	1	0	0	0	0
		7	0.1	0	4	3	0	0	0
		2	0.5	0	0	1	1	0	0
		1	1.0	0	1	0	0	0	0
		4	5.0	0	0	1	0	2	1
5	<1+ in 0.1	2	0.1	0	0	2	0	0	0
		1	1.0	0	0	1	0	0	0
		2	5.0	0	0	0	0	1	1
29	0.1	7	0.1	0	2	1	2	0	2
		1	0.5	0	0	0	1	0	0
		7	1.0	0	0	1	3	1	2
		14	5.0	0	1	2	3	3	5
4	0.5	1	0.1	0	0	0	1	0	0
		1	0.5	0	0	1	0	0	0
		2	1.0	0	0	0	0	0	2
11	1.0	4	1.0	0	0	1	1	0	2
		7	5.0	0	0	1	2	1	3
3	5.0	3	5.0	0	0	0	3	0	0
88		88		16	14	15	17	8	18

* At least 1+ growth except where <1+ is indicated.

medium. It may also be seen that, although 27 of the 88 patients were in the negative or 1-plus category, this low colony count of tubercle bacilli was directly correlated with the absence of isoniazid-resistant organisms.

The maximum bacteriologic conversion to negative for tubercle bacilli was obtained at the end of the eighth week of treatment when sputum from 19 of the 88 isoniazid-treated patients failed to produce any growth of *M. tuberculosis* on suitable culture media. It may be noted, moreover (*see* table 8), that at approximately this time acid-fast bacilli with significant isoniazid resistance were first isolated from many of the patients. The bacteriologic picture worsened after this period, as may be seen in tables 6 and 7. This appeared to be directly related to the continued appearance of tubercle bacilli resistant to more than 0.1 γ of isoniazid per ml. of medium.

In table 8 the development of isoniazid resistance of tubercle bacilli isolated

TABLE 7

APPEARANCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI RELATED TO COLONY COUNT AT THE END OF TWELVE AND THIRTY-TWO WEEKS OF TREATMENT

PATIENTS WITH BACILLI RESISTANT TO ISONIAZID AT END OF TWELVE WEEKS OF TREATMENT		END OF THIRTY-TWO WEEKS OF TREATMENT							
				Growth* on Medium without Isoniazid					
Number of Patients	Growth* on Medium with Isoniazid	Number of Patients	Growth* on Medium with Isoniazid	Negative	<1+	1+	2+	3+	4+
	γ per ml.		γ per ml.						
21	0	20	0	18	1	1	0	0	0
		1	1.0	0	1	0	0	0	0
18	0.1	1	N.R.	0	1	0	0	0	0
		3	0.1	1	2	0	0	0	0
		3	0.5	0	1	0	1	1	0
		3	1.0	0	0	0	2	1	0
		8	5.0	0	3	2	2	1	0
4	0.5	2	0.5	0	0	1	1	0	0
		1	1.0	0	0	0	0	0	0
		1	5.0	0	0	0	0	1	0
15	1.0	4	1.0	0	1	1	0	2	0
		11	5.0	0	0	2	0	5	4
30	5.0	2	1.0	0	1	0	1	0	0
		28	5.0	0	0	5	7	8	8
88		88		19	11	11	14	19	13

* At least 1+ growth except where <1+ is indicated.

from the 88 patients during the eight months of treatment is summarized. It will be seen that during the first three weeks no isoniazid-resistant organisms were found. A small percentage of the patients discharged tubercle bacilli resistant to more than 0.1 γ of drug per ml. of medium between the sixth and eighth weeks, and a significant number of patients discharged bacilli resistant to 1.0 or 5.0 γ of isoniazid per ml. of medium after eight weeks of treatment with the drug. This is of interest because as mentioned above the optimal bacteriologic results were obtained at the end of eight weeks of treatment and thereafter an increase in the count of *M. tuberculosis* colonies began to appear.

Comment: The clinician might find it expedient to continue treatment with isoniazid after bacilli resistant to only 0.1 γ per ml. have appeared. In this situation, however, the probability is slight that reversal of infectiousness will occur. Actually, as seen in the present series, when at first only a few colonies grew on the media containing 0.1 γ per ml., the tendency was toward a greater culture count in succeeding specimens. Therefore, it seems that the appearance of resistance to even 0.1 γ per ml. plays an important role bacteriologically, even though continued treatment may be justified. It is of interest to mention that

TABLE 8

PATTERN OF APPEARANCE OF ISONIAZID-RESISTANT STRAINS OF M. TUBERCULOSIS ISOLATED FROM 88 PATIENTS TREATED WITH THE DRUG FOR THIRTY-TWO WEEKS

WEEKS OF TREATMENT	RESISTANCE IN γ PER ML. OF MEDIUM									
	Number of Patients with No Drug-Resistant Bacilli		0.1		0.5		1.0		5.0	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Pretreatment	88	100	0		0		0		0	
1	88	100	0		0		0		0	
2	88	100	0		0		0		0	
3	86	97.7	2	2.3	0		0		0	
4	74	84.1	14	15.9	0		0		0	
5	56	63.6	32	36.4	0		0		0	
6	43	48.9	42	47.8	1	1.1	1	1.1	1	1.1
7	39	44.4	46	52.3	0		1	1.1	2	2.2
8	36	41.1	34	38.6	4	4.5	11	12.5	3	3.4
12	21	23.9	18	20.5	4	4.5	15	17.0	30	34.1
32	23	25.1	3	3.4	5	5.7	10	11.4	47	53.4

increased resistance to the drug (to 1.0 γ or more) and the resultant increase in colony count (to 1-plus or higher) were present whenever a worsening was noted on roentgenography in the group of 88 patients here reported (6).

SUMMARY

One thousand three hundred and twenty-seven concentrated sputum specimens from 88 ambulatory tuberculous patients treated with isoniazid for eight months were examined simultaneously by microscopy and by culture. It was found that, when the number of tubercle bacilli in the concentrated specimens was small, the usefulness of microscopy was negligible, for 77 per cent of such microscopic examinations of smears were negative when the cultures were positive for *M. tuberculosis*. Only 2.4 per cent of all of the specimens revealed acid-fast bacilli on microscopy and no tubercle bacilli on culture.

An evaluation method of reporting culture results expressed in terms of 4-plus, 3-plus, 2-plus, and less than 1-plus growth was presented to show a direct correlation between numbers of *M. tuberculosis* colonies on culture and numbers of acid-fast bacilli on microscopy of aliquots of the same concentrated specimens.

The appearance of isoniazid-resistant strains of *M. tuberculosis* in patients under treatment with the drug was noted at intervals during the eight-month treatment period. The colony growth evaluation was used successfully to measure the bacteriologic progress of treated patients.

SUMARIO

Los Procedimientos de Laboratorio Empleados para Diagnóstico en los Ejemplares de Esputo Procedentes de Tuberculosos Ambulantes Tratados con Isoniacida

Concentrados de 1,327 ejemplares de esputo procedentes de 88 tuberculosos ambulantes tratados con isoniacida durante ocho meses fueron examinados simultáneamente con

técnicas de frotes y de cultivos. Observóse que, cuando era pequeña la cantidad de bacilos tuberculosos en los concentrados, la eficacia del método de los frotes era menospreciable, dado que 77 por ciento de los mismos eran negativos cuando los cultivos eran positivos. Solamente 2.4 por ciento de todos los ejemplares dieron bacilos ácidorresistentes positivos en los frotes y negativos en los cultivos.

Ofrécese un método de justipreciación para presentar los resultados de los cultivos, expresándolos en términos de colonias de 4 más, 3 más, 2 más, 1 más y menos de 1 más para demostrar una correlación directa entre los números de colonias de *M. tuberculosis* en los cultivos y los números de bacilos ácidorresistentes en los frotes de concentrados idénticos.

A ciertos intervalos, durante el perfodo de ocho meses de tratamiento, notóse formación de isoniacido-resistencia de parte de los *M. tuberculosis* aislados de los enfermos bajo tratamiento. Para medir el mejoramiento bacteriológico de los sujetos tratados se usó con éxito la justipreciación de la proliferación de las colonias.

RESUME

Procédés diagnostiques de laboratoire utilisés pour les spécimens de crachats provenant de tuberculeux traités par l'isoniazide

Treize cent vingt-sept spécimens concentrés de crachats, prélevés chez 88 tuberculeux non alités et traités par l'isoniazide pendant huit mois, furent examinés simultanément par l'examen direct et par la culture. Il a été observé que, lorsque le nombre de bacilles tuberculeux était bas, l'efficacité de la technique d'examen direct était négligeable car 77% des lames étaient négatives alors que les cultures étaient positives. Ce n'est que dans 2,4% de la totalité des spécimens que les examens directs et les cultures se sont montrés positifs pour les bacilles acido-résistants.

Les auteurs offrent une méthode d'évaluation pour noter les résultats des cultures qui s'exprime en termes de 4-plus, 3-plus, 2-plus, 1-plus. Des déterminations de croissance de moins de 1-plus ont été présentées pour démontrer la corrélation directe entre le nombre de colonies de *M. tuberculosis* obtenues en culture et le nombre de bacilles acido-résistants dans les étalements de concentrés identiques.

Le développement de la résistance à l'isoniazide de *M. tuberculosis* isolés, chez des malades traités par ce produit, fut observé périodiquement au cours des huit mois de traitement. L'évaluation du développement de colonies fut utilisée avec succès pour mesurer l'amélioration bactériologique, chez les malades en traitement.

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Case Reports

DIAGNOSTIC AIDS IN HISTOPLASMOSIS[1]

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INTRODUCTION

Within recent years histoplasmosis has been recognized with such increased frequency that it is no longer considered of sufficient rarity to warrant reporting each new case which is discovered. Nevertheless, substantiated diagnoses of the disease are difficult to obtain. In the disseminated form of histoplasmosis, it is often possible to isolate the etiologic agent from blood and bone marrow. In the pulmonary form, especially when cavitation is present, yeast cells are occasionally identified in sputum on direct smear or, more often, cultures of the organism may be obtained from sputum. It is the case of diffuse pneumonitis caused by *Histoplasma capsulatum* which presents the most perplexing challenge to the diagnostician. Clinically and roentgenographically the picture resembles practically all other pneumonias. Even if the physician is astute enough to include histoplasmosis in his differential diagnosis, he will find it difficult to substantiate or reject this diagnosis either by positive evidence or by exclusion. Accordingly, the following case is reported as it illustrates several aids in the diagnosis of histoplasmosis.

CASE REPORT

A. A., a white male 59 years of age, was admitted to a local hospital on March 30, 1953 with a complaint of fever, malaise, and night sweats of two weeks' duration. There was no cough, expectoration, or hemoptysis. The onset was acute three days after cleaning out his chicken house preparatory to laying down a wooden floor.

On physical examination the patient appeared nervous, apprehensive, and anxious. His temperature was 100°F., pulse was 80, and respirations were slightly increased in rate. No localizing signs were elicited in the lungs. The examination was essentially noncontributory.

Laboratory data: The leukocyte count was 5,300 per cu. mm.; erythrocyte count, 5,220,000 per cu. mm.; hemoglobin, 14.35 gm; and the erythrocyte sedimentation rate was 35 mm. per hour. The urine was normal. A roentgenogram of the chest revealed a diffuse, fine nodular infiltration of miliary type throughout both lung fields. The skin reaction to tuberculin was negative to 0.01 mg. of Old Tuberculin (OT), first test, and negative to 1.0 mg. of OT on a subsequent test. The histoplasmin skin reaction was negative to 0.1 cc. of 1:1,000 Lilly Histoplasmin Concentrate (CT-189). A precipitin test for histoplasmosis was positive 1:4, and a complement fixation test was negative. Sputum smears and culture were negative for tubercle bacilli and *H. capsulatum* on two occasions.

Course: On symptomatic treatment the patient improved progressively. The temperature after the day of admission never rose above 99°F. After eight days of hospitalization the patient was discharged as improved. A second histoplasmin skin test done on May 14, 1953 showed a 2-plus reaction. Subsequent chest roentgenograms showed progressive clearing of the miliary infiltration, and by August 18, 1953 there was complete resolution without residual scarring or calcification.

Serologic tests were repeated at intervals with the following results:

[1] From the Tennessee Department of Public Health, Williamson County Tuberculosis Study, Franklin, Tennessee.

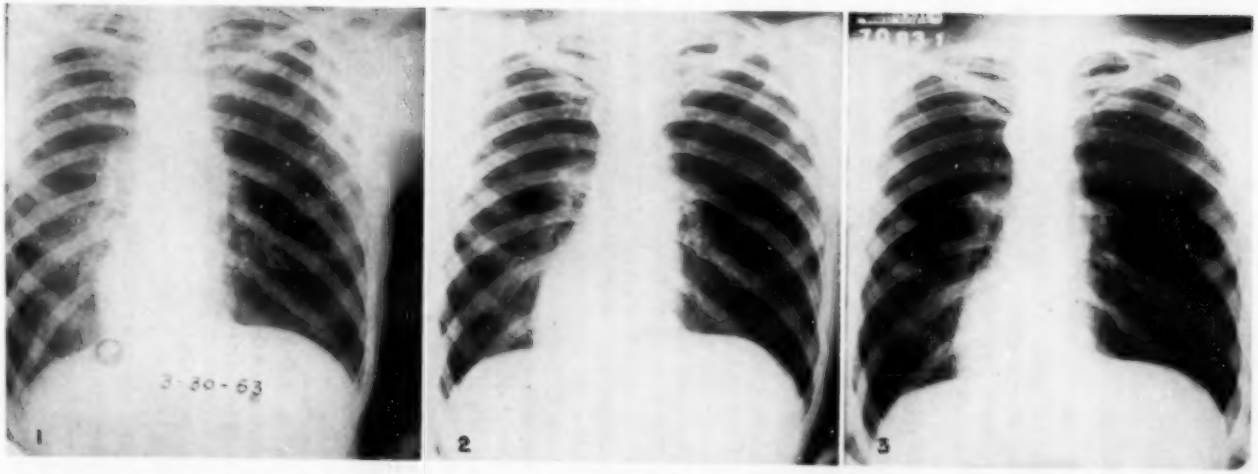

FIG. 1. Postero-anterior chest roentgenogram taken March 30, 1953. Diffuse, fine nodular infiltration throughout both lung fields.

FIG. 2. Postero-anterior chest roentgenogram taken May 14, 1953. Some clearing of the fine nodular infiltration noted on film of March 30, 1953.

FIG. 3. Postero-anterior chest roentgenogram taken August 18, 1953. Complete clearing of lesions noted on previous films (figures 1 and 2).

May 14, 1953: Precipitin test—positive undiluted; complement fixation test—doubtful

July 16, 1953: Precipitin test—positive 1:160; complement fixation test—positive 1:8

August 18, 1953: Precipitin test—positive 1:160; complement fixation test—positive 1:80

December 28, 1953: complement fixation test—positive 1:40

COMMENT

The purist may argue that a diagnosis of histoplasmosis is justified only if the etiologic agent is recovered from discharges or tissues of the patient. If this rigid criterion were to be accepted exclusively, only a fraction of the occurrences of the disease would be diagnosed. Since it is now known that nonfatal histoplasmosis does exist and that instances of the disease are by no means uncommon, the clinician should be ready to accept presumptive evidence in the absence of positive identification of the fungus.

In the instance here reported, several significant findings may be mentioned: first, the conversion of the histoplasmin test from negative to positive within two months after the onset of the illness; second, the evidence of an increase in and subsequent waning of the immunologic response as manifested by the precipitin and complement fixation tests for histoplasmosis. Especially in an endemic area, such evidence is highly suggestive. Since this kind of evidence has been accepted by clinicians and immunologists for other diseases, it should be acceptable for histoplasmosis even though immunologists are not yet agreed on which test is most sensitive, which antigen is best, or how to interpret results.

The patient's roentgenogram presented still another point of interest. The initial chest film was suggestive of a miliary tuberculosis. However, the patient was not critically ill, and his tuberculin reaction was negative to 1.0 mg. of OT. In the relatively short period of four months the diffuse nodular infiltration of the lungs resolved completely without residual scarring or calcification. No antimicrobial therapy was administered during the illness. Treatment was entirely symptomatic. The spontaneous recovery of this patient is further proof, if more were needed, that not all histoplasmosis is fatal.

In several previously reported studies (1, 2) it has been noted that there appears to be some association between excreta of fowl and histoplasmosis. The history in this case indicated that the patient probably was exposed to the etiologic agent while he was cleaning out his chicken house. On August 11, 1953, samples of soil were obtained from inside the chicken house. *H. capsulatum* was isolated from this soil. Although recovery of the organism from the presumed source of exposure cannot of itself be regarded as proof of the diagnosis of histoplasmosis, it is nevertheless an additional piece of supporting evidence in this case.

Acknowledgments

We wish to express our gratitude to Dr. Walter Pyle of Franklin, Tennessee, for making his clinical records of this patient available to us. We should like to acknowledge the contributions of Dr. Joseph H. Schubert, Serology Unit, and Dr. Libero Ajello, Mycology Unit of the Communicable Disease Center, Public Health Service, who were respectively responsible for the precipitin tests and the mycological study of the soil.

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Notes

A TRISODIUM PHOSPHATE TRANSPORT-DIGESTION METHOD OF PROCESSING SPUTUM AND GASTRIC SPECIMENS FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS

The increasing number of sputum and gastric specimens that were being submitted for culture of *M. tuberculosis* to the diagnostic laboratory of the Bureau of Laboratories, New York City Department of Health, prompted a search for a less time-consuming method of digestion than the widely used 4 per cent sodium hydroxide-hydrochloric acid procedure. This was done after taking into consideration the following peculiar conditions under which this laboratory operates:

1. A large number of specimens are received daily from the twenty-three widely scattered chest clinics of the Bureau of Tuberculosis.
2. All specimens are cultured after a delay of twenty-four to seventy-two hours, mostly at room temperature, because of transportation.
3. Laboratory space and personnel are sharply limited.

Preliminary investigations of a variety of previously described procedures finally led to a study of the possibility of using trisodium phosphate as a digestant during the period of transportation so that the specimens would be completely digested and ready for centrifuging immediately upon arrival at the laboratory.

In this connection, two separate but related studies were carried out. First, a series of controlled cultural experiments were conducted to determine the optimal concentration of trisodium phosphate to use under the conditions described. Second, a comparative study was made of the relative efficacy of the trisodium phosphate and the sodium hydroxide methods of digestion on routine clinic specimens.

Effect of various concentrations of trisodium phosphate on the viability of small numbers of M. tuberculosis in sputum and gastric specimens: Phosphate buffer (pH 7.0) suspensions of 25 freshly isolated cultures of *M. tuberculosis* were standardized to an opacity equivalent to 3×10^8 bacterial units per ml. by use of the McFarland standard. Tenfold dilutions of the emulsions were then prepared, varying from a 10^{-1} to a 10^{-8} dilution of the original emulsion, and 1 ml. of each culture dilution was added to 9 ml. of fresh, negative pooled sputum, in duplicate sets. To one set of sputum culture tubes were added 10 ml. of 10 per cent trisodium phosphate[1], and to the duplicate set 10 ml. of 23 per cent trisodium phosphate. Thus, eight duplicate sets were prepared for each of the 25 strains of *M. tuberculosis*. The tubes were then left at 22° to 24°C. for the duration of the experiment. At twenty-four-hour intervals during the first seven days and once again at the end of fourteen days, the duplicate sets of tubes containing the sputum culture-trisodium phosphate mixture were centrifuged and 0.25 ml. of each precipitate cultured on two slants of medium.[2] The culture slants were incubated at 37°C. and examined for the presence of colonies of *M. tuberculosis* at weekly intervals for four weeks. Observations on the digestion and contamination of each specimen were made at the end of each twenty-four-hour period.

To 39 ml. of each of 50 fresh negative gastric washings was added 1 ml. of a phosphate buffer (pH 7.0) suspension of *M. tuberculosis* previously standardized to contain 15,000 bacterial units per ml. A different strain of *M. tuberculosis* was added to each gastric speci-

[1] Reagent grade.

[2] One tube of Jensen's modification of Löwenstein's medium and one tube of Peizer-Schechter agar medium.

men. After each gastric culture mixture was well shaken, it was subdivided into four parts of 10 ml. each. Each of the four portions was then treated as follows: one portion was processed by the usual 4 per cent sodium hydroxide-hydrochloric acid method, and this served as a control; the three other portions were mixed with 10 ml. of 23 per cent, 15 per cent, and 10 per cent trisodium phosphate, respectively. The gastric culture-trisodium phosphate mixtures were then left at 22° to 24°C. for a total of seventy-two hours. At the end of forty-eight hours and again at seventy-two hours, two samples of 1 ml. of the well-shaken suspension were removed from each of the 150 tubes of gastric culture-trisodium phosphate mixtures and spread evenly on Peizer-Schechter agar plates. The seeded plates were sealed to prevent drying, incubated at 37°C., and examined for the growth of *M. tuberculosis* colonies at weekly intervals for four weeks. Observations on the digestion and contamination of each specimen were made at the end of each twenty-four-hour period.

Both 23 per cent and 10 per cent trisodium phosphate were equally effective in the digestion of the sputum specimens during the one to fourteen days of exposure at room temperature. Similarly, 23 per cent, 15 per cent, and 10 per cent trisodium phosphate were all equally effective in the digestion of the gastric specimens exposed for twenty-four to seventy-two hours at room temperature. No contamination was encountered throughout these experiments.

In table 1 the relative toxicity of 10 per cent and 23 per cent trisodium phosphate on small numbers of *M. tuberculosis* suspended in sputum specimens at room temperature for one to fourteen days is indicated.

In table 2 the relative toxicity of 10 per cent, 15 per cent, and 23 per cent trisodium phosphate on small numbers of *M. tuberculosis* suspended in gastric washings at room temperature for forty-eight to seventy-two hours is indicated.

It is apparent from the tables that 10 per cent trisodium phosphate is superior to 23 per cent for maintaining the viability of *M. tuberculosis* in both sputum and gastric specimens.

Because of the results, it was decided to use 10 per cent trisodium phosphate as a

TABLE 1

AVERAGE AMOUNT* OF GROWTH OF VARYING NUMBERS OF M. TUBERCULOSIS FROM 25 DIFFERENT STRAINS ADDED TO POOLED NEGATIVE SPUTUM AND ALLOWED TO REMAIN EXPOSED TO 10 PER CENT AND 23 PER CENT TRISODIUM PHOSPHATE AT 22° TO 24°C. FOR FOURTEEN DAYS

NUMBER OF DAYS EXPOSED	FINAL DILUTIONS OF EXPOSED SUSPENSIONS OF M. TUBERCULOSIS, STARTING WITH AN ORIGINAL ONE CONTAINING 3×10^{-8} BACTERIAL UNITS PER ML.															
	10^{-1}		10^{-2}		10^{-3}		10^{-4}		10^{-5}		10^{-6}		10^{-7}		10^{-8}	
	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4	10 per cent Na_3PO_4	23 per cent Na_3PO_4
1	4+	4+	4+	3+	4+	2+	4+	2+	2+	1+	1+	5 col.	50 col.	0	8 col.	0
2	4+	4+	4+	3+	4+	2+	3+	2+	2+	50 col.	1+	0	35 col.	0	5 col.	0
3	4+	4+	4+	2+	4+	2+	3+	1+	2+	30 col.	1+	0	60 col.	0	9 col.	0
4	4+	4+	4+	2+	4+	1+	3+	1+	2+	25 col.	1+	0	50 col.	0	5 col.	0
5	4+	3+	4+	2+	4+	1+	2+	1+	2+	15 col.	1+	0	30 col.	0	8 col.	0
6	4+	3+	4+	1+	4+	2 col.	2+	0	2+	0	1+	0	52 col.	0	3 col.	0
7	4+	2+	4+	50 col.	4+	0	2+	0	1+	0	50 col.	0	20 col.	0	0	0
14	4+	1+	4+	0	4+	0	1+	0	1+	0	35 col.	0	5 col.	0	0	0

* Quantitative values: 4+: confluent growth; 3+: discrete growth; 2+: 100–150 colonies; 1+: 50–100 colonies; Col.: colonies.

TABLE 2

AVERAGE AMOUNT* OF GROWTH OF SMALL NUMBERS† OF M. TUBERCULOSIS FROM 50 DIFFERENT STRAINS ADDED TO 50 NEGATIVE GASTRIC SPECIMENS AND ALLOWED TO REMAIN EXPOSED TO VARIOUS CONCENTRATIONS OF TRISODIUM PHOSPHATE FOR 48 TO 72 HOURS AT 22° TO 24°C.

PERIOD OF EXPOSURE IN HOURS	TREATED WITH 4 PER CENT NaOH-HCl	EXPOSED TO $Na_3PO_4 \cdot 12H_2O$		
		23 Per Cent	15 Per Cent	10 Per Cent
Control	2+	–	–	–
48	–	1+	2+	2+
72	–	25 colonies	1+ to 2+	2+

* Quantitative values: 1+: 50 to 100 colonies per agar plate; 2+: 100 to 200 colonies per agar plate.

† Approximately 15,000 bacterial units were added to 40 ml. of each gastric specimen, which was then divided into four equal portions.

TABLE 3

COMPARATIVE RESULTS OF CULTURES FOR M. TUBERCULOSIS FROM SPUTUM AND GASTRIC SPECIMENS DIGESTED BY EITHER 4 PER CENT SODIUM HYDROXIDE-HYDROCHLORIC ACID OR 10 PER CENT TRISODIUM PHOSPHATE

TYPE OF SPECIMEN	NUMBER OF DUPLICATE SETS	CONTAMINATED SPECIMENS		SPECIMENS NEGATIVE BY BOTH METHODS	POSITIVE SPECIMENS							
		NaOH Method	Na_3PO_4 Method		Either Method or Both		Both Methods		Only by NaOH Method		Only by Na_3PO_4 Method	
					Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Sputum	591	14	3	353	238	100	204	85.7	13	5.5	21	8.8
Gastric	522	18	0	467	55	100	48	87.3	2	3.6	5	9.1
Total	1,113	32	3	820	293	100	252	86.0	15	5.1	26	8.9

transport-digestant for both sputum and gastric specimens collected from clinic patients for the comparative study that follows.

Comparative study of the trisodium phosphate and the sodium hydroxide methods of digestion of sputum and gastric specimens from chest clinic patients: Two different types of sputum bottles, combined with tape to form a single unit, were given to clinic patients with known or suspected pulmonary tuberculosis with instructions to collect specimens as follows:

1. Expectorate during the first twenty-four hours into the bottle[3] containing 30 ml.[4] of 10 per cent trisodium phosphate.
2. Expectorate during the succeeding twenty-four hours into the empty bottle.
3. Then deposit the combined set of specimens promptly at a collecting station.

[3] Ordinary glass may react with the trisodium phosphate to produce an inactive precipitate which does not interfere with the processing of the specimen. Pyrex glassware does not noticeably have this effect.

[4] This volume was arbitrarily chosen. Subsequent observations indicated that, with this volume of 10 per cent trisodium phosphate, marked variations in the volume of sputum or gastric washings did not significantly affect the cultural results.

A total of 591 sets of combined specimens were examined for the purpose of this study. The untreated or control specimens (twenty-four to forty-eight hours old) were processed by the 4 per cent sodium hydroxide-hydrochloric acid method. The trisodium phosphate specimens (forty-eight to seventy-two hours old) were merely shaken on a Shaer or Kahn heavy-duty shaking machine for ten minutes, centrifuged at 1,800 to 2,000 r.p.m. for twenty minutes, and the precipitate then used to inoculate tubes of Jensen-Löwenstein and Peizer-Schechter media. The tubes were then incubated and examined in the usual manner.

The routine twenty-four-hour-old gastric specimens from 522 clinic patients were processed as follows:

1. One half of each specimen was treated promptly on arrival at the laboratory by the 4 per cent sodium hydroxide-hydrochloric acid method.
2. The other half of each specimen was mixed with an equal volume of 10 per cent trisodium phosphate and left at 22° to 24°C. for sixty to seventy-two hours. The specimens were then shaken, centrifuged, and the precipitate cultured in the same manner as described for the similarly treated sputum specimens.

The results of the cultures for *M. tuberculosis* from the combined sets of sputum and gastric specimens are noted in table 3. It is at once apparent that neither the trisodium phosphate nor the sodium hydroxide-hydrochloric acid method has any particular advantage over the other as far as cultural results are concerned. The trisodium phosphate method, however, is distinctly superior to the sodium hydroxide-hydrochloric acid method in preventing contamination.

The cultural results of this comparative study indicate no advantage of the widely used 4 per cent sodium hydroxide-hydrochloric acid method of digestion over the trisodium phosphate transport-digestion method. Because the latter procedure requires considerably less use of laboratory time, personnel, and space, it would seem to be the preferred one for sputum and gastric specimens which must be transported to a central laboratory for processing twenty-four to seventy-two hours after collection.

For this reason, the Bureau of Laboratories has adopted the trisodium phosphate transport-digestion method for all specimens submitted from clinic patients.

In summary: Controlled cultural experiments indicated that 10 per cent trisodium phosphate was the most effective concentration for use as a transport-digestant for sputum and gastric specimens. The use of 10 per cent trisodium phosphate as a transport-digestant for specimens twenty-four hours or longer in transit to the laboratory was just as effective as the 4 per cent sodium hydroxide-hydrochloric acid method of digestion as far as cultural results were concerned. It had the distinct advantage, however, of preventing contamination more effectively and of being much less time-consuming.

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THE ANTITUBERCULOUS ACTIVITY OF PYRAZINAMIDE IN VITRO AND IN THE GUINEA PIG

Several reports have described favorable results with pyrazinamide alone in both experimental and clinical tuberculosis.[1-9] It was therefore considered of interest to record observations on the *in vitro* effect as well as the *in vivo* activity of this drug in the experimental animal.

The *in vitro* effect of pyrazinamide against the H37Rv strain of *M. tuberculosis* was tested in three different types of medium, namely, Tween®-albumin medium, Proskauer and Beck synthetic medium, and Proskauer and Beck synthetic medium with 10 per cent horse serum. Complete inhibition of growth for fourteen days was not obtained with concentrations of the drug as high as 200 γ per ml. (the highest concentration used). A slight inhibition of growth by 100 and 200 γ of drug per ml. was noted in the two media without serum. Three different lots of the drug[10] (7-8036, 7-8362, and 7-8627) gave the same results.

The effect of pyrazinamide against acute tuberculosis in guinea pigs was evaluated in two separate experiments, the results of which are presented in tables 1 and 2. In both, the guinea pigs were infected subcutaneously with the H37Rv strain of *M. tuberculosis* and the drug was administered as outlined in the tables. Four different lots of the drug were used, three in the first experiment and one in the second. It may be seen that the results obtained with pyrazinamide were not striking; the treated guinea pigs in each experiment showed very little difference from the control untreated group. For comparison, the findings in a group of pigs treated with isoniazid are indicated in table 2.

Other workers have found that pyrazinamide has only slight *in vitro* activity. Solotorovsky and associates[9] reported that 150 γ per ml. was the inhibitory concentration

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[10] The pyrazinamide used in all the described experiments was generously supplied by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

TABLE 1

EFFECT OF PYRAZINAMIDE AGAINST ACUTE TUBERCULOSIS IN GUINEA PIGS

Infection: H37Rv strain of *M. tuberculosis*, 0.1 mg. (dry weight) subcutaneously. Treatment started 21 days after infection. All survivors killed 75 days after infection.

TREATMENT	NUMBER DEAD WITHIN 75 DAYS/TOTAL NUMBER OF ANIMALS	AVERAGE DEGREE OF TUBERCULOSIS*				
		Lungs	Liver	Spleen	Lymph Nodes	Total
Controls	0/10	2.2	2.75	2.75	3.15	10.85
Pyrazinamide,† 50 mg. twice daily, orally	1/10	1.45	1.8	2.7	3.25	9.2

* Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of from 0 to 4 according to the amount of gross tuberculous disease. Maximum total for each animal was, therefore, 16.

† Aldinamide ®, Lederle, Lot Nos. 7-8036, 7-8277, and 7-8362.

TABLE 2

EFFECT OF PYRAZINAMIDE AGAINST ACUTE TUBERCULOSIS IN GUINEA PIGS

Infection: H37Rv strain of *M. tuberculosis*, 0.1 mg. (dry weight) subcutaneously. Treatment started 30 days after infection. All survivors killed 100 days after infection.

TREATMENT	NUMBER DEAD WITHIN 100 DAYS/TOTAL NUMBER OF ANIMALS	AVERAGE DEGREE OF TUBERCULOSIS*				
		Lungs	Liver	Spleen	Lymph Nodes	Total
Controls	3/12 (25 per cent)	2.7	3.6	3.6	3.6	13.5
Pyrazinamide,† 55 mg. twice daily, orally	4/10 (40 per cent)‡	1.6	2.1	2.75	3.1	9.6
Isoniazid, 5 mg. per day, intramuscularly	0/10	0.1	0.2	0.95	2.3	3.55

* *See* table 1.

† Aldinamide ®, Lederle, Lot. No. 7-8627.

‡ Two died of unknown cause after 8 and 10 days of treatment, respectively, and were not included in the tabulation.

against the H37Rv strain in Tween®-albumin medium. Tarshis and Weed[11], using many strains of *M. tuberculosis* freshly isolated from patients, were able to demonstrate only partial inhibition with 100 and 1,000 γ of pyrazinamide per ml. on blood and Herrold's medium, and no inhibition even with 1,000 γ per ml. on solid American Trudeau Society medium. Dessau and associates[12] found that all of their strains of tubercle bacilli isolated from patients were completely inhibited for fourteen days by 100 γ per ml. in Tween®-albumin medium, but they qualified this by stating that there was considerable variation from one experiment and from one strain to the other.

In regard to the effect of pyrazinamide on guinea pig tuberculosis, Dessau and asso-

[11] TARSHIS, M. S., AND WEED, W. A., JR.: Am. Rev. Tubere., 1953, *67*, 391.

[12] DESSAU, F. I., BURGER, F., YEAGER, R. L., AND KULISH, M.: Am. Rev. Tubere., 1952, *65*, 635.

ciates[3] have reported the finding of "moderate but definite antituberculous activity." They apparently obtained as good results in acute tuberculosis with 50 mg. of pyrazinamide orally as with 15 mg. of streptomycin subcutaneously per day. Again, this average result was qualified by the finding of "very considerable variation of response to the same dosage with different samples of the drug." In two survival experiments, these writers reported no beneficial effect from pyrazinamide in one. In the second, a rather meager difference in the T_{50} was found among three groups: 62 days in the controls, 82 days with 30 mg. of drug daily subcutaneously, and 50 to 87 days with 75 mg. daily in the diet.

It will be noted that four different lots of pyrazinamide were used in the experiments reported herein.[13] The drug used in the second experiment was supplied by the manufacturer in answer to a specific request for a batch with proved antituberculous activity.

The described clinical benefits of pyrazinamide when used alone in man and its antituberculous effect in the mouse as shown by the microbial enumeration technique[14] occur during the first few weeks of the treatment period and in both man and the mouse may be only temporary. It is possible that the treated guinea pigs in the present experiments might have shown some difference from the controls if they had been killed earlier in the course of the infection. Then, however, one would have expected some difference to persist at least until the seventy-fifth day of infection unless the drug had stimulated the growth of the tubercle bacillus *in vivo* or, through its toxicity, altered host resistance. Such alteration could induce a more rapid spread of disease in the treated animal than in the controls, as has been observed by the present investigators with other drugs.

In summary, pyrazinamide was found to have only slight *in vitro* activity against the H37Rv strain of *M. tuberculosis* in concentrations as high as 200 γ per ml. and to produce very little beneficial effect in two separate experiments when administered orally to tuberculous guinea pigs in dosage of 50 to 55 mg. twice daily (approximately 145 mg. per kg. per day).

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TRUDEAU, NEW YORK

WILLIAM STEENKEN, JR.
EMANUEL WOLINSKY

February 23, 1954

[13] The results of the second experiment were mentioned briefly in a previous report: STEENKEN, W., JR., AND WOLINSKY, E.: Tr. Nat. Tuberc. A., 1952, p. 425.

[14] MCDERMOTT, W., ORMOND, L., MUSCHENHEIM, C., DEUSCHLE, K., MCCUNE, R. M., JR., AND TOMPSETT, R.: Am. Rev. Tuberc., 1954, *69*, 319.

THE CULTURE OF TUBERCLE BACILLI FROM RESECTED LUNG LESIONS

A Comparison of Bovine Albumin and Physiologic Saline as Diluents of Tissue Homogenates in the Recovery of Tubercle Bacilli by Culture and Animal Inoculation

The inability to grow tubercle bacilli which are readily visible in stained preparations and pathologic sections of residual necrotic foci obtained from patients after prolonged antituberculous drug therapy[1-4] raises certain questions. Are the techninques[5] now in use for the preparation of material for culture and animal inoculation adequate, or are the stainable intact-appearing bacilli that are now recoverable actually dead?

Accordingly, a re-examination of present-day techniques has been undertaken by the Sunmount and Trudeau laboratories as well as by other groups of investigators[6] to determine whether the methods employed are adequate for demonstrating the viability and virulence of tubercle bacilli in such residual lesions.

Dubos has suggested that in the necrotic lesion the absorption by the tubercle bacillus of a growth inhibitor may prevent multiplication of the organisms until the inhibitory agent is removed or neutralized. The exhaustion of an essential metabolite which must be supplied in a proper environment to permit multiplication of the tubercle bacillus has also been postulated[7-8]. In the same articles, reference is made to *in vitro* experiments which indicates that the accumulation of organic acids, particularly lactic acid, during the anaerobic metabolism of inflammatory cells may cause rapid death of tubercle bacilli. It has also been brought out by Middlebrook[9] that the possible release of toxic agents, such as lactic acid, during the homogenization of resected lesions may kill the tubercle bacilli present prior to culture or inoculation of guinea pigs, and the addition of bovine albumin to the specimen as a possible detoxifying or neutralizing agent was suggested.

It is the purpose of this paper to report findings relative to the effect of adding fraction V bovine albumin (Armour) to necrotic material in preparation of the specimen prior to the attempted recovery of tubercle bacilli by culture or animal inoculation.

Large discrete necrotic lesions, usually 1 cm. in diameter or larger, were enucleated from the resected lung specimen under aseptic conditions. These were divided into two

[1] D'Esopo, N., Bernstein, S., Decker, A. M., Raleigh, J. W., and Steenken, W., Jr.: Transactions of the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, Atlanta, Ga., February, 1953, p. 229.

[2] Steenken, W., Jr.: Transactions of the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, Atlanta, Ga., February, 1953, p. 268.

[3] American Trudeau Society: Am. Rev. Tubere., 1953, *68*, 477.

[4] Steenken, W., Jr., and Wolinsky, E.: Sixth International Congress of Microbiology, Rome, 1953, vol. I, p. 314.

[5] Diagnostic laboratory methods as revised by the Veterans Administration Laboratory Committee following the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, June, 1953.

[6] Hall, W. H., and Gleason, D. F.: Transactions of the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, Atlanta, Ga., February, 1953, p. 219.

[7] Dubos, R. J.: Transactions of the Eleventh Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, St. Louis, Mo., January, 1952, p. 234.

[8] Dubos, R. J.: Transactions of the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, Atlanta, Ga., February, 1953, p. 271.

[9] Middlebrook, G.: Personal communication.

TABLE 1

COMPARISON OF RESULTS BY THE TWO TECHNIQUES FOR DEMONSTRATION OF VIABLE TUBERCLE BACILLI

TYPE OF CASE	NUMBER OF LESIONS EXAMINED	POSITIVE FINDINGS SALINE HOMOGENATES		POSITIVE FINDINGS ALBUMIN HOMOGENATES		NEGATIVE FINDINGS BY BOTH TECHNIQUES
		Culture	Guinea Pig	Culture	Guinea Pig	
A. "Target point" cases						
1. Closed lesions (positive on microscopy)	12	0	0	0	0	12
2. Closed lesions (negative on microscopy)	5	1	1	1	1	4
B. "Non-target point" cases						
1. Open lesions	2	2	2	2	2	0
2. Closed lesions (positive on microscopy)	19	9	9	10	10	7
3. Closed lesions (negative on microscopy)	1	0	0	0	0	1

TABLE 2

THE EXTENT OF DIFFERENCES OBTAINED WITH THE TWO TECHNIQUES FOR DEMONSTRATION OF VIABLE TUBERCLE BACILLI

LESION	SALINE HOMOGENATES		ALBUMIN HOMOGENATES	
	Culture	Guinea Pig	Culture	Guinea Pig
1	1 Col.	−	−	−
2	−	−	2 Col.	+
3	5 Col.	+	−	+
4	−	−	2 Col.	−
5	−	+	10 Col.	+

Col. = colonies.

equal portions. One portion was treated as follows: The tissue was placed in a sterile glass tissue grinder (Tenbroeck) and 4 ml. of 2.5 per cent fraction V bovine albumin in 1 per cent saline were added before grinding. The albumin solution was prepared by adding 5 gm. of the albumin to 100 ml. of 2 per cent saline, sterilized by passing through a Seitz filter, and the enzyme activity inactivated by heating at 56°C. for one hour. The solution was then diluted with an equal volume of sterile distilled water, bringing the albumin concentration down to 2.5 per cent in 1 per cent saline. The other portion served as a control. To this, 4 ml. of sterile physiologic saline were added, and the tissue was ground as above. Within thirty minutes after obtaining the enucleated lesion, the necrotic material contained therein was homogenized in the presence of the bovine albumin or normal saline, cultured, and inoculated into guinea pigs. From each of the two types of homogenates (saline and bovine), 2 ml. were inoculated into one guinea pig, the inoculum being divided exactly between the right and left inguinal regions; and 2 ml. were planted in a total of five tubes of American Trudeau Society solid medium.

Cultures were inspected for growth at weekly intervals and kept for a minimum of

eight weeks before discarding. All animals were tuberculin tested prior to inoculation and at six-week intervals thereafter until they were sacrificed at the end of three months.

Table 1 represents the comparison of the results obtained by culture and animal inoculation of the saline and albumin homogenates prepared from 39 lesions obtained from 19 pulmonary resections. Of these, 17 lesions were obtained from 11 "target point"[10] cases, and the remaining 22 lesions were obtained from "non-target point" cases. Only one of the "target point" lesions proved to be positive for *M. tuberculosis* by both techniques on culture and guinea pig inoculation. Of even more significance was the failure to obtain any positive results with either technique from 12 of these lesions, although they proved to be positive for acid-fast bacilli on direct microscopy.

Not unexpectedly, the lesions obtained from "non-target point" cases were positive for tubercle bacilli on culure or animal inoculation in more than 50 per cent of the lesions studied.

The differences obtained with the two techniques are illustrated in table 2. It is immediately apparent that the minor discrepancies which occurred in 5 instances are purely chance findings which are well known to occur in comparative studies of this type and are not related to the superiority of one technique over the other.

The amount of growth obtained by culture and the degree of tuberculosis observed in the animals were essentially the same for the saline and bovine albumin homogenates of the specimens which contained acid-fast bacilli on direct microscopy.

In summary: Bovine albumin fraction V may be used as a diluent for homogenization of tuberculous lesions in preparation for culturing for tubercle bacilli. It is not, however, superior to physiologic saline for the preparation of tissue homogenates as determined by its ability to stimulate the recovery of tubercle bacilli by culture or to produce progressive tuberculosis in guinea pigs.

The discrepancies are of the type usually encountered in comparative experiments of this kind and are not considered to be related to the superiority of one technique over the other.

The ability of bovine albumin to detoxify or neutralize the inhibitory substances in the lesions or released during homogenization which would interfere with the viability and virulence of the tubercle bacillus could not be verified by the techniques employed in this study.

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January 15, 1954

[10] "Target point" cases refer to those patients receiving prolonged chemotherapy before resectional surgery and whose pulmonary disease is characterized by (*1*) stable roentgenograms, (*2*) closure of all cavities, and (*3*) sputum cultures persistently negative for tubercle bacilli for at least three months preoperatively. "Non-target point" cases refer to those patients receiving prolonged chemotherapy before resectional surgery but failing to meet these three criteria.

[11] Present address: Veterans Administration Hospital, West Haven, Connecticut.

Correspondence

METHODS OF PNEUMOTHORAX INDUCTION

To the Editor of The American Review of Tuberculosis:

The recent editorial on the induction of pneumothorax justifies mentioning some studies performed by Dr. George Ewart of this hospital and Dr. Irving Chofnas, now at the Houston Veterans Administration Hospital. With the trend away from pneumothorax, the studies were not completed or reported.

Before the studies, we were using for induction of pneumothorax either a 16-or 19-gauge, blunt, side-opening needle with trochar, or the latter without trochar. The incidence of collapse greater than planned was sufficient to justify routine analgesia orders on all inductions (the incidence from memory was approximately 20 per cent). It appeared that the blunt needles could not be incriminated, but rather the trauma was produced by a freely moving lung against the trochar or against the sharp needle used for preliminary anesthesia infiltration. Since the exact thickness of the chest wall could not be accurately estimated, there seemed to be no way to avoid the implacement of these sharp instruments at too deep a level in a fair percentage of cases, and a patient could not be expected to hold his breath for a sufficient period of time to prevent movement of the underlying lung against the sharp point.

Recognizing these obstacles, it was decided to attempt deliberate and rapid lung puncture, throughout which the patient could hold his breath, thus avoiding laceration and excessive loss of air into the pleural space. The patient was asked to hold his breath and, without anesthesia, a sharp 19-gauge needle was stabbed through the pleura and immediately withdrawn. After four hours there usually was sufficient space to proceed with a routine refill. The method was used enough to indicate its superiority over the original method of pneumothorax induction. Two untoward incidents caused its abandonment. One apprehensive patient breathed deeply as the needle was introduced and a 50 per cent collapse was produced in a few minutes with no further collapse. Except for the use of the analgesia administered routinely to patients under the old method, no treatment was required. In an additional patient, on the fourth attempt at pneumothorax induction a small high pressure pocket was produced which required one aspiration of air for relief of symptoms.

In patients with extensive disease and adherent pleura, the quick stab with the 19-gauge needle into an immobile lung had a lower incidence of hemoptysis when pneumothorax induction was a failure than the so-called standard techniques.

We then studied the effect of a quick stab with a sharp 23-gauge needle into an immobile (theoretically) lung as a method of pneumothorax induction. No excessive collapse was produced, but failures were high. The average case required three trips to the treatment room on successive days. After the stab with the 23-gauge needle, several hours elapsed before an expiration chest roentgenogram was taken. If a fine line of pneumothorax was seen on the wet film, the patient was returned immediately to the treatment room for a routine refill. If no pneumothorax developed, the induction was attempted again on another day. Our third, and last, untoward incident demonstrated that a pneumothorax present on an expiration film on the day before could absorb enough to produce trouble at a refill the next morning.

Although demonstrated as safe, the 23-gauge needle stab into an immobile lung developed into a time-consuming nuisance. Perhaps abandonment of its safety features by stabbing three or four times on the initial attempt should have been the next step, but we abandoned the study because of the attitude of other physicians. We discussed the methods used on numerous occasions. Most physicians agreed with us in principle, but were unwilling to use a deliberately traumatic method on their patients. A few were violently opposed.

Our third method has not been proved by any large series of cases because of the trend away from pneumothorax. The infiltration of the tissues with a local anesthetic through a sharp 23-gauge needle is again used. Once through the skin, the patient holds his breath each time the needle is advanced. After each advancement, the plunger of the syringe is pulled back immediately. If the operator notes a minute inflow of air from the needle into the syringe, or if the operator receives a sensation that the needle tip has passed through a layer of tissue which may be pleura, the needle is withdrawn before the patient is allowed to breathe. The next steps are similar to those with the planned stab of the lung by the 23-gauge needle.

If the procaine infiltration is accomplished without obvious penetration of the pleura, the blunt 19-gauge side-opening needle is used. There is an elaborate procedure for introducing this needle which has no bearing on the discussion except for the abandonment of the guiding trochar, which eliminates inadvertent trauma to the lung by the sharp edge of the trochar.

Our last method seems to be a safe method and reconciles the differences between the traumatic and nontraumatic methods of pneumothorax induction, but it has not been tested on a large series of cases.

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Respectfully submitted,
W. E. ROYE

February 26, 1954

LIMITATIONS OF THE GUINEA PIG TEST

To the Editor of the American Review of Tuberculosis:

A word of caution seems in order concerning the use of guinea pig tests in the tuberculosis laboratory. Their use for typing acid-fast organisms and for primary isolation of tubercle bacilli is well established. It is generally held that the guinea pig test is nearly as sensitive as, or more sensitive than, the culture method for primary isolation, and the guinea pig is the first, if not the final, court of appeal in determination of virulence of a questionable acid-fast organism. Some designate as saprophytes organisms which are not virulent for guinea pigs. Work recently reported and some older observations cast serious doubt on the wisdom of this practice.

Griffith[1] long ago found that many cultures evidently pathogenic for man (mostly lupus strains) lacked typical virulence for guinea pigs, and the same is true of other acid-fast organisms associated with superficial lesions in man. The possibility that avian bacilli may produce disease in man has never been disproved, and it has long been established that this species has very little virulence for guinea pigs. More recently, inoculation of guinea pigs—even silicotic ones[2]—with material from closed cavitary lesions has failed to demonstrate viable bacilli when carefully prepared cultures from the same material have

[1] GRIFFITH, A. S.: Tubercle, 1924, 5, 569.

[2] STEENKEN, W., JR.: Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, February, 1954 (to be published).

been positive[3]. Tubercle bacilli, in becoming resistant to isoniazid, lose their virulence for guinea pigs[4]. There is no evidence, however, that tubercle bacilli are necessarily avirulent for man. Finally, recent trends toward surgical therapy and more universal and adequate cultural methods in the laboratory seem to have resulted in showing up another inadequacy of the guinea pig. Chromogenic or otherwise atypical organisms have been found in diseased resected lung tissue[5-7]. Such chromogens have very little, if any, virulence for guinea pigs, but their association with morbid human tissue in the absence of other demonstrable pathogens, plus other evidence, strongly suggests possible pathogenicity for man.

Guinea pigs then may fail in reflecting virulence for man in the case of acid-fast bacteria from lupus and other superficial lesions; in the case of avian bacilli; the bacilli of closed cavitary lesions, of isoniazid-resistant strains; and for other strains of mycobacteria, for some of which there is increasing evidence of virulence for man. Guinea pigs are no less valuable than they ever were, but the belief that they are infallible arbiters of human virulence seems to be unjustified.

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Sincerely yours,
ERNEST H. RUNYON

VIRULENCE OF ISONIAZID-RESISTANT TUBERCLE BACILLI

To the Editor of The American Review of Tuberculosis:

The letters by D. A. Mitchison (Am. Rev. Tuberc., 1954, *69*, 640) and Sheila M. Stewart (Am. Rev. Tuberc., 1954, *69*, 641) in the April, 1954, issue of THE AMERICAN REVIEW OF TUBERCULOSIS emphasize the fact that our conclusions regarding the virulence of tubercle bacilli recovered from patients treated with isoniazid were based on the study of cultures containing mixtures of drug-sensitive and drug-resistant bacilli and may, therefore, be misleading. Mitchison's results, on the other hand, are derived from the study of cultures artificially manipulated in order to eliminate the organisms resistant to less than the minimal inhibitory concentration of the culture as a whole. With 13 such strains exhibiting varying degrees of isoniazid resistance, he demonstrates nicely a "very definite relation between virulence and degree of resistance."

We were well aware of the fact that our "crude" primary isolated strains and sputum concentrates contained mixtures of drug-sensitive and drug-resistant organisms. We purposely chose to study such strains and concentrates in this early work because of a desire to investigate this phenomenon in the population of bacilli just as they came from the patient with as little manipulation as possible. From the study of 33 such "crude" strains we stated that the "correlation between the degree of *in vitro* resistance and the degree of virulence for guinea pigs is not clear-cut," and that "the phenomenon of loss of virulence of tubercle bacilli for guinea pigs is related to other factors in addition to the development of isoniazid resistance." We did not mean, however, to imply that the two were entirely unrelated.

[3] HOBBY, G., *et al.*: Thirteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, February, 1954 (to be published).

[4] KARLSON, A. G., AND IKEMI, Y.: Proc. Staff Meet., Mayo Clin., 1954, *29*, 119.

[5] BUHLER, V. B., AND POLLAK, A.: Am. J. Clin. Path., 1953, *23*, 263.

[6] HALL, W., AND ERLANDSON, H.: Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, Navy, February, 1954 (to be published).

[7] TIMPE, A., AND RUNYON, E. H.: Thirteenth Conference on Chemotherapy of Tuberculosis, of the Veterans Administration, Army, Navy, February, 1954 (to be published).

It may eventually be proved that every cell resistant to a certain concentration of isoniazid in a culture of tubercle bacilli will be nonvirulent for guinea pigs. Even assuming this to be true, we feel that our conclusions probably would still be justified because:

1. We have observed the same phenomenon of loss of virulence for guinea pigs in cultures of tubercle bacilli that had become resistant when exposed to certain drugs other than isoniazid, as pointed out in our original paper (Am. Rev. Tuberc., 1953, *68*, 548).
2. The explanation for the continued virulence of certain of these "crude" freshly isolated strains which are highly resistant as determined by the *in vitro* isoniazid-sensitivity test (the fact that they may still contain a considerable proportion of sensitive cells) is a logical one. What then is the explanation for the finding of very much reduced virulence for guinea pigs in others which are only slightly or moderately resistant to isoniazid by routine sensitivity tests when it can be demonstrated that only a very small proportion of the individual cells in these cultures are resistant to as much as 1γ per ml.? We have seen several such strains. Certainly, in the relatively large inoculum used, there should be enough sensitive cells in such mixtures of organisms to produce progressive disease in guinea pigs, *if only the isoniazid-resistant cells had lost virulence.*
3. Certain unexpected results are coming to light as our study progresses which indicate that degree of resistance to isoniazid is not the sole factor involved in the loss of virulence for guinea pigs of tubercle bacilli from patients taking isoniazid. For instance, cultures of tubercle bacilli were isolated from four separate areas in a resected lung from a patient who had received isoniazid as well as streptomycin and PAS. All four cultures were highly resistant to streptomycin and PAS, but isoniazid resistance varied from slight (only partial resistance to 0.2γ and no growth on 1.0γ and 5.0γ per ml.) to high (good growth in 5γ per ml.). Despite these very gross differences in the percentage of sensitive and resistant organisms making up the total population of each of these four cultures, they all exhibited the same very slight virulence for guinea pigs.

It seemed to us that the most immediate problem to be settled was whether "the results of these virulence tests in animals can be applied to man," and we, therefore, proceeded along the lines of correlating the clinical course of the patient receiving isoniazid with the results of serial virulence tests performed on the population of tubercle bacilli recovered from his sputum. The study of this phenomenon in so-called pure cultures, or artificially manipulated cultures, is certainly of great interest to the bacteriologic investigator and a necessary corollary to the first problem, but let us not forget that tubercle bacilli in the body of the patient are actually "crude mixtures" of organisms and not pure cultures in which all of the sensitive cells have been eliminated.

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Sincerely yours,
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EMANUEL WOLINSKY

April 20, 1954

COUNTING CHAMBERS FOR THE ENUMERATION OF TUBERCLE BACILLI

To the Editor of the American Review of Tuberculosis:

Dr. G. S. Wilson, Director of Public Health Laboratory Service, London, who has great experience in the use of counting chambers for bacteria, has expressed as his opinion[1]

[1] Personal communication, 1951.

that such a chamber should be circular and closed so as to avoid evaporation, and has emphasized the high performance of the Helber chamber. During a visit in London, I was given the opportunity to use such a chamber in an attempt to count the bacterial units in BCG vaccine. The chamber was the only one of its kind available in England at the time and was transported with great care from one scientific institution to another according to need. Since then, data have been published[2] demonstrating the apparent superiority of the circular, closed chamber over those that are now commercially available. One of these, manufactured in this country, bears the name of Helber, although in important aspects if differs from the chamber described by that author.[3]

Recently, during attempts to enumerate the bacterial units in cultures of BCG and R_1 in Dubos Tween®-albumin medium, the disadvantages of the commercially available and currently used counting chambers have again become evident[4]. These chambers are rectangular in shape and open at both ends, permitting evaporation of fluid from the chamber. Possibly for this reason, currents are present in the fluid so that the bacilli to be counted are found to move at varying speed across the ruled floor of the chamber. This unabated movement is present even fifteen to twenty minutes after the filling of the chamber. Another factor which may reduce the precision of the chambers mentioned is the matte, ground glass surface on which the coverslip rests. With such a surface, it is impossible to observe Newton's rings, perhaps the only reliable indicator of close and consistent contact between the chamber and the coverslip. The correct depth of the cell is only 0.02 mm., and it must be expected that even the slightest error of contact would seriously disturb the count.

Having been informed that a circular, closed chamber for bacterial counts is at present not available, we have recently started to employ an old model Thoma Zeiss hemocytometer. This chamber has a circular well and is completely closed by the coverslip, resting against a polished surface which allows the formation of Newton's rings. The height of the chamber was ground down from the original 0.10 mm. to 0.02 mm.[5] to make it suitable for bacterial counts. This chamber, a near approach to the original Helber chamber, has proved to be satisfactory. The distribution of the bacilli in the chamber is stable for hours, and they show no movement except the Brownian which, in the case of tubercle bacilli, is to the advantage of the observer. With the now available counting chambers, we have found a reasonably accurate enumeration of units of tubercle bacilli in Dubos medium to be virtually impossible. With the circular, closed chamber, however, it is a comparatively easy procedure which can be repeated with a satisfactory degree of precision.

I believe it would be to the advantage of those engaged in enumeration of acid-fast bacteria if the Helber chamber, according to the original description, were again made available.

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North Carolina Sanatorium
McCain, North Carolina

Yours sincerely
Edward F. Krohn

March 27, 1954

[2] Krohn, E. F.: Acta tuberc. Scandinav., 1952, Supplement 30, 38.

[3] Helber, E.: Deutsche Arch. klin. Med., 1904, *81*, 317.

[4] This study was made possible by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[5] C. A. Hausser and Son, Philadelphia.

Books

THORACIC SURGERY. Second Edition. By Richard M. Sweet. Pp. 381, W. B. Saunders Co., Philadelphia and London, 1954, Price, $10.00.

Dr. Sweet has revised the first edition of his book to provide for the description of newer techniques and certain alterations in others since they were first described. The general outline of the book is the same as the first edition, stressing surgical anatomy, operative techniques, and postoperative care. Its being brought up to date continues to make it a good guide for the graduate student of thoracic surgery, as well as a reference for the thoracic surgeon himself. It is recommended particularly for its clarity and brevity and also for its careful attention to detail.

The subject matter is arranged on a regional anatomic basis, beginning with the surgical anatomy of the thorax, general technical considerations and thoracic incisions, and proceeding through operations on the chest wall, the pleural cavity, the lungs, the mediastinum, the esophagus, the diaphragm, and abdominal operations performed through thoracic incisions. Newer techniques have been added, prominent among which are those involving cardiovascular surgery.

The magnificent illustrations originally done by Dr. George Rodriguez Arroya have been altered very little, although certain deletions and additions have been made in conformity with the revision of the second edition.

Dr. Sweet's book again stresses the operative techniques and does not profess to discuss thoracic diseases as such.

DONALD L. PAULSON
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BAKTERIOLOGISCHE NÄHRBÖDEN. By Lothar Hallmann. Pp. 252, Georg Thieme Verlag, Stuttgart, 1953.

This volume forms the counterpart to a book by the same author entitled "Bacteriology and Serology—Selected Methods for the Bacteriological and Serological Laboratory." The contents are divided into two parts. In the first part (64 pages) are discussed various basic ingredients and the general methods of preparation of culture media as well as the procedures of pH determination and sterilization. The second part comprises 177 pages and deals with the various culture media used for the determination of biochemical activities ("fermentation reactions") of bacteria and for the isolation and identification of the more important pathogenic bacteria, fungi, and protozoa. Included in the volume, which is thumb-indexed, are author index, subject index, and 52 illustrations.

The text is clearly written and the material concisely presented. The printing is excellent and the cover of the book is washable. The volume is up-to-date, as the more recently described culture media, such as the Tween®-albumin medium of Dubos, are included. This volume should be of great value to all bacteriologists in whose laboratories culture media are prepared from basic ingredients. There is less need for such a tome in this country, where the majority of culture media are available in dehydrated form from commercial sources.

ERWIN NETER
Buffalo, New York

ACUTE PULMONARY EDEMA. Modern Medical Monographs No. 10. By Mark D. Altschule. Pp. 68, Grune & Stratton, New York, 1954, Price, $3.50.

This is a small book, scholarly, informative, and well written. The author's stated purpose is "to relate both the manifestations and the treatment of acute pulmonary edema to what is known about certain physiologic phenomena." Such is done with more thorough and concise coverage than this reviewer has yet seen.

The book opens with review of the usual clinical manifestations of pulmonary edema, proceeds with the basic physiologic considerations thereof, the clinical conditions in which pulmonary edema may be found, with attempt at explanation of its occurrence in each, and review of available therapy.

Chief emphasis is put on bringing existing data to bear on just what train of circumstances, in any given instance, initiated the edema. This results in clarifying discussion on a number of controversial points, i.e., how important is pulmonary capillary pressure in precipitating pulmonary edema in patients with mitral stenosis; why are uremics so prone to develop the syndrome; and similar subjects.

Commonly held misconceptions in pulmonary edema pathogenesis are pointed out. The pharmacologic and physiologic basis of the various modes of therapy are set forth.

There is much still to be learned on the subject. It is a virtue of this monograph that it is quick to point out where knowledge is lacking and more work needed.

One of the harder jobs in clinical medicine is keeping up to date our physiologic understanding of what we see. We are fortunate when the work thus involved is made easier for us by books such as these.

CARL WIERUM
New York, New York

BOOKS RECEIVED

TUBERCULOSIS IN CHILDHOOD AND ADOLESCENCE. By F. J. Bentley, S. Grzybowski, and B. Benjamin. Pp. 259, with illustrations, National Association for the Prevention of Tuberculosis, Tavistock House North, London, 1954, Price, $5.00.

STREPTOCOCCAL INFECTIONS. Edited by Maclyn McCarty. Symposium held at the New York Academy of Medicine, February 25 and 26, 1953. Pp. 218, Columbia University Press, New York, 1954, Price $5.00.

WINE AS FOOD AND MEDICINE. By Salvatore P. Lucia. Pp. 149, The Blakiston Company, Inc., New York and Toronto, 1954, Price, $3.00.

NOTES

INTERIM SCIENTIFIC SESSION OF THE AMERICAN TRUDEAU SOCIETY

The American Trudeau Society will hold an Interim Scientific Session at the Hotel New Yorker, New York City, on November 17, 1954, from 9 a.m., to 5 p.m. The meeting will be open to physicians interested in pulmonary diseases in internal medicine, surgery, and pediatrics, and to scientists in allied disciplines.

The program will consist of a series of presentations on various aspects of pulmonary diseases from the clinical and laboratory standpoints. A detailed program will be announced at a later date.

Persons interested in participating in the program may submit abstracts of material to be presented for selection for the program. Such abstracts should not exceed 200 words in length, and should be typewritten double-spaced, and submitted in quadruplicate, The deadline for submission is October 1. The abstracts should be addressed to:

Dr. William B. Tucker, Chairman
Interim Scientific Session Program Committee
Veterans Administration Hospital
54th Street and 48th Avenue South
Minneapolis 17, Minnesota

It is anticipated that notification of selection for participation will be sent to authors submitting abstracts by October 15.

Titles may be submitted on any subject in pulmonary disease, from either a laboratory or clinical standpoint. Not more than twelve minutes will be allotted for presentation. Adequate time for discussion of each paper has been planned. Manuscripts need not be submitted for publication. Investigative work in progress may be reported, but papers should represent original investigative work. Inquiries about further details will be welcome.

THE JAMES ALEXANDER MILLER FELLOWSHIP FOR RESEARCH IN TUBERCULOSIS AND ALLIED PROBLEMS

The New York Tuberculosis and Health Association announces that a fellowship will be available from July 1, 1955 to June 30, 1956.

The Miller Fellowship is designed to support a qualified investigator in medical or biological sciences who will devote full time to a research project in Tuberculosis or allied problems. Assurance must be provided that the applicant will be acceptable in the laboratory or clinic of his choice; and that he will be provided with the facilities necessary for the pursuit of the work.

The annual stipend will be: $5,000. Consideration will be given for renewal of the Fellowship for one or more years.

Applications should be submitted in duplicate by November 1, 1954. Application forms will be supplied on request. Address—The New York Tuberculosis and Health Association, 386 Fourth Avenue, New York 16, N. Y. Awards will be announced December 15, 1954.

FORTIETH SESSION OF THE TRUDEAU SCHOOL OF TUBERCULOSIS

Thirty-six doctors from six foreign countries and thirteen states attended the 40th annual session of the Trudeau School of Tuberculosis which ended its four-week course on June 25.

Among the foreign countries represented were Canada, Australia (Tasmania), Thailand, Japan, Korea, and Brazil. Some of the doctors were in private practice; others were public health officials, staff doctors at various hospitals, and instructors in medicine in universities. Six were from the Department of Mental Hygiene of New York State; five from the Veterans Administration; four from the U. S. Navy; two from the U. S. Air Force; and one each from the U. S. Public Health Service and the American Korean Foundation.

Sessions of the School were held at Trudeau Sanatorium and other divisions of the Trudeau-Saranac Institute, as well as Ray Brook State Tuberculosis Hospital, Sunmount Veterans Administration Hospital, Variety Clubs-Will Rogers Hospital, Sanatorium Gabriels, Stony Wold Sanatorium, and the Saranac Lake Rehabilitation Guild.

The faculty was composed of physicians, surgeons, and scientists from the Saranac Lake area as well as several guest lecturers. Among the latter were: Dr. Paul T. Chapman, Detroit; Dr. Ronald V. Christie, London, England; Dr. Richard V. Ebert, Chicago; Dr. James J. Feffer, Washington, D. C.; Dr. Jerome Hartz and Dr. Theodore E. Woodward, Baltimore; Dr. Robert H. High, Philadelphia; Dr. Edward M. Kent, Pittsburgh; Dr. Anthony J. Lanza, Dr. John K. Lattimer, Dr. James E. Perkins, and Dr. Kenneth W. Smith, New York; Dr. Donald B. Miller, Burlington, Vermont; Dr. Laurence L. Robbins, Boston; Mr. Ivan Sabourin, Montreal; Dr. Oscar A. Sander, Milwaukee; and Dr. David T. Smith, Durham, North Carolina.

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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Doniklan, M., and Pikula, D.: Am. Rev. Tuberc. 67:808, 1953.

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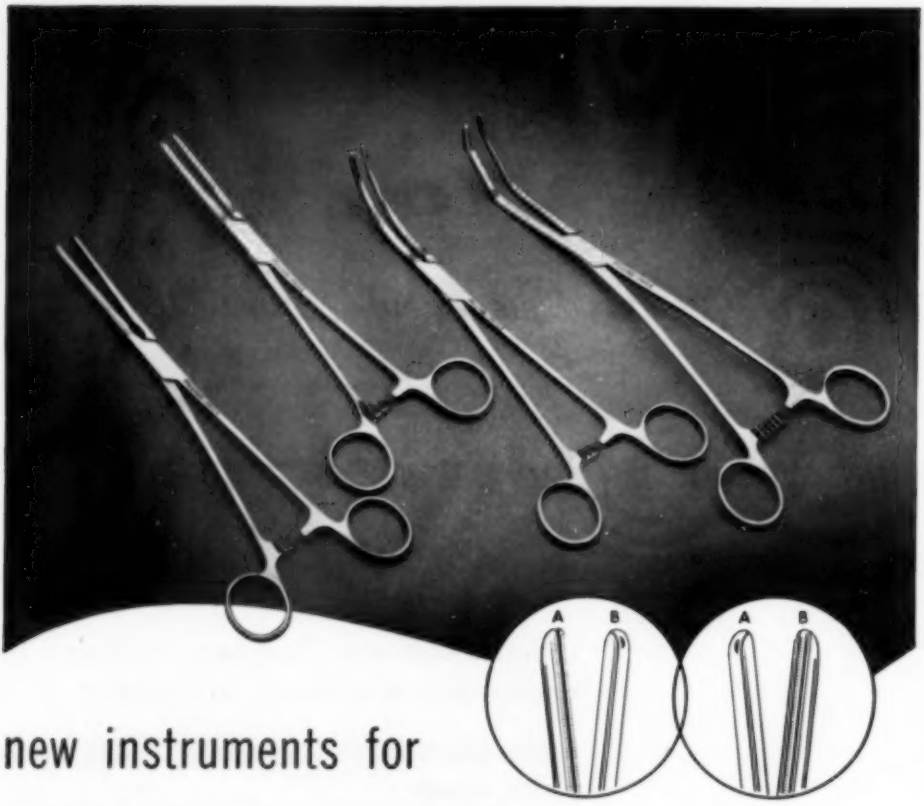

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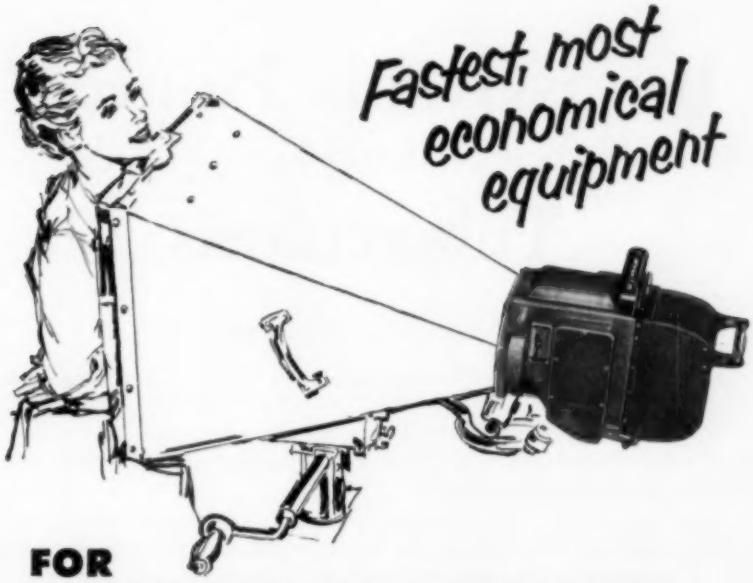

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